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EXHIBIT D

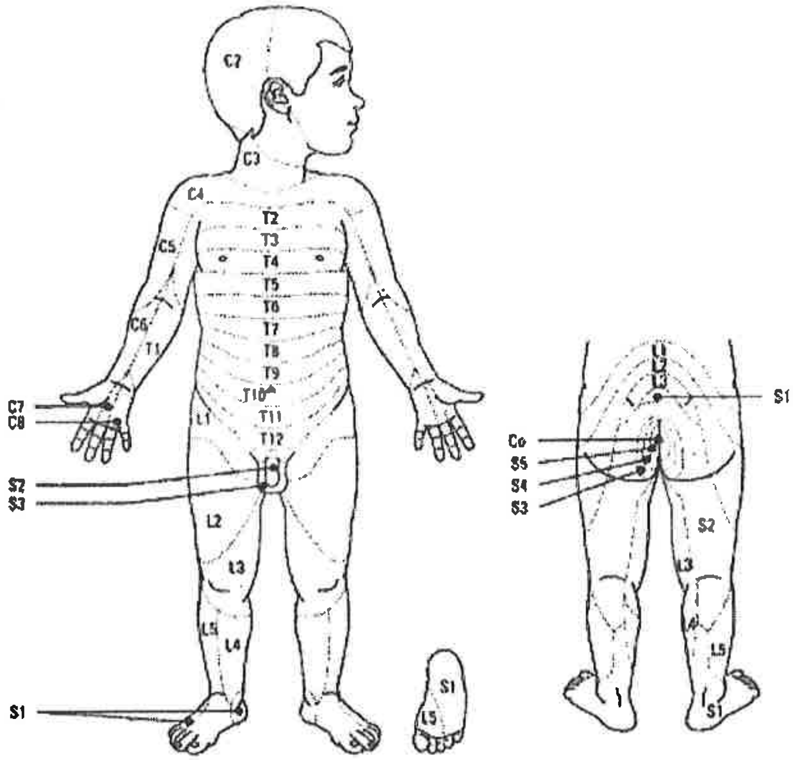
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EXHIBIT E

Effect of Serologic Status and Cesarean Delivery on Transmission Rates of Herpes Simplex Virus From Mother to Infant

Zane A. Brown, MD
Anna Wald, MD, MPH
R. Ashley Morrow, PhD
Stacy Selke, MS
Judith Zeh, PhD
Lawrence Corey, MD

Context Neonatal herpes most commonly results from fetal exposure to infected maternal genital secretions at the time of delivery. The risk of transmission from mother to infant as it relates to maternal herpes simplex virus (HSV) serologic status and exposure to HSV in the maternal genital tract at the time of labor has not been quantified. Furthermore, no data exist on whether cesarean delivery, the standard of care for women with genital herpes lesions at the time of delivery, reduces HSV transmission.

Objective To determine the effects of viral shedding, maternal HSV serologic status, and delivery route on the risk of transmission of HSV from mother to infant.

Design Prospective cohort of pregnant women enrolled between January 1982 and December 1999.

Settings A university medical center, a US Army medical center, and 5 community hospitals in Washington State.

Patients A total of 58362 pregnant women, of whom 40023 had HSV cultures obtained from the cervix and external genitalia and 31663 had serum samples tested for HSV.

Main Outcome Measure Rates of neonatal HSV infection.

Results Among the 202 women from whom HSV was isolated at the time of labor, 10 (5%) had neonates with HSV infection (odds ratio [OR], 346; 95% confidence interval [CI], 125-956 for neonatal herpes when HSV was isolated vs not isolated). Cesarean delivery significantly reduced the HSV transmission rate among women from whom HSV was isolated (1 [1.2%] of 85 cesarean vs 9 [7.7%] of 117 vaginal; OR, 0.14; 95% CI, 0.02-1.08; $P=.047$). Other risk factors for neonatal HSV included first-episode infection (OR, 33.1; 95% CI, 6.5-168), HSV isolation from the cervix (OR, 32.6; 95% CI, 4.1-260), HSV-1 vs HSV-2 isolation at the time of labor (OR, 16.5; 95% CI, 4.1-65), invasive monitoring (OR, 6.8; 95% CI, 1.4-32), delivery before 38 weeks (OR, 4.4; 95% CI, 1.2-16), and maternal age less than 21 years (OR, 4.1; 95% CI, 1.1-15). Neonatal HSV infection rates per 100000 live births were 54 (95% CI, 19.8-118) among HSV-seronegative women, 26 (95% CI, 9.3-56) among women who were HSV-1–seropositive only, and 22 (95% CI, 4.4-64) among all HSV-2–seropositive women.

Conclusion Neonatal HSV infection rates can be reduced by preventing maternal acquisition of genital HSV-1 and HSV-2 infection near term. It can also be reduced by cesarean delivery and limiting the use of invasive monitors among women shedding HSV at the time of labor.

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THE PREVALENCE OF GENITAL and neonatal herpes in the United States continues to rise.[1] About 1.6 million new herpes simplex virus (HSV) 2 infections are acquired yearly and more than 2% of women seroconvert to HSV-2 during pregnancy.[2,3] Although previous studies suggest that neonatal HSV infection is often a consequence of HSV acquisition in late pregnancy,[3-10] the risk of transmission of HSV-1 or HSV-2 from mother to infant as it relates to maternal HSV serologic status and exposure to HSV in the maternal genital tract at the time of labor has not been quantified. Furthermore, cesarean delivery has been the standard of care for more than 3 decades for women with genital herpes lesions at the time of delivery, but no data exist on whether this procedure reduces HSV transmission, and some obstetrical authorities have discussed abandoning this practice.[11-16]

To evaluate the risk factors for the transmission of HSV from mother to infant, women in labor had a genital viral culture obtained and serum samples saved for retrospective analysis of HSV-1 and HSV-2 serologic status.

METHODS

Participants, Setting, and Procedures

This study was carried out at University of Washington Medical Center, Se-

Author Affiliations are listed at the end of this article.
Corresponding Author and Reprints: Zane A. Brown, MD, Box 356460, Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195-6460 (e-mail: zbrown@u.washington.edu).

attle, between January 1982 and December 1999, at 5 community hospitals in Seattle between January 1984 and October 1990, and at Madigan Army Medical Center, Tacoma, Wash, between August 1990 and September 1997. Viral cultures were obtained from women in labor by swabbing the vulva, perineal, and perianal areas. A separate swab was used to obtain a sample from the upper vagina and cervix.[3,8] If genital lesions were identified on examination during delivery and the peripartum period, cultures were obtained from the lesion and placed in a separate vial of transport media.[17] Only women who had cultures obtained within 48 hours of delivery were included in the analysis. Prophylactic acyclovir therapy was not used during pregnancy, at delivery, or during the immediate postpartum period for any of the exposed infants.[11,12,18] Maternal HSV serologic status was determined from samples collected at delivery or at obstetrical antepartum testing. Culture results were reported to the attending physician, as were the results of HSV serologic tests after the test became available for clinical use. Written informed consent was obtained for the portions of the study protocol not included in routine clinical care according to the University of Washington Institutional Review Board.

Data Collection

Routine demographic and delivery data were collected on deliveries at all hospitals from 1989-1999 and at the University of Washington during the entire 18-year study period. Delivery room charts were reviewed for history and signs of genital herpes, duration of membrane rupture, and route of delivery in women from whom HSV was isolated at delivery and in women whose infants developed neonatal herpes.

Laboratory Methods

Herpes simplex virus cultures and HSV DNA detection by polymerase chain reaction were performed as previously described.[19,20] Serum samples obtained at the time of labor were stored and tested for antibodies to HSV-1 and HSV-2 by Western blot assay following delivery.[21]

Definitions and Statistical Analysis

The serologic and virologic classification of HSV status at delivery was defined as previously published.[3,8,22] Women with primary-episode genital herpes were defined as having HSV-1 or HSV-2 isolated from genital secretions without having concurrent HSV antibodies. A nonprimary first-episode infection was defined as HSV-2 isolated from genital secretions of a woman with only HSV-1 antibodies, or HSV-1 isolated from a woman with only HSV-2 antibodies. Reactivation HSV-1 or HSV-2 was present when the virus isolated from genital secretions was the same type as antibodies present in the serum at the time of labor. Symptomatic shedding was defined as the isolation of HSV when genital lesions were noted on entering the labor suite, and subclinical shedding as isolation of HSV in the absence of genital lesions.[17,22,23]

Relative risks were assessed by computing odds ratios (ORs) with 95% confidence intervals (CIs). *P* values were obtained using 2-sided χ^2 or Fisher exact tests, with $P<.05$ considered statistically significant. Adjusted ORs were obtained from bivariate logistic regression. In examining risk factors for neonatal herpes, we adjusted for only 1 confounder at a time because of the small number of infected neonates and the correlation among the risk factors. We estimated rates of neonatal infection as a function of maternal serologic status at the 2 hospitals (University of Washington and Madigan) at which routine serologic testing for HSV was performed. These were derived with the assumption that the infants of women with known serologic status were a random sample of all infants delivered, stratified by hospital and time. Because serologic status was known for most women, the estimated denominators of the rates were very precisely determined, with coefficients of variation of 1%. Standard errors for neonatal infection rates by maternal serologic status were derived by the delta method, taking into account the uncertainty associated with the small number of cases as well as the estimated serologies. As the SEs derived by the delta method differed from SEs based on known denominators only in the fifth significant digit, CIs were based on exact CIs for binomial proportions. Statistical analyses were carried out using SPSS, version 8.0 (SPSS Inc, Chicago, Ill), and S-PLUS, version 3.1 (Insightful Corp, Seattle, Wash). The same study team carried out the study, including data management and analysis, for the entire period.

RESULTS

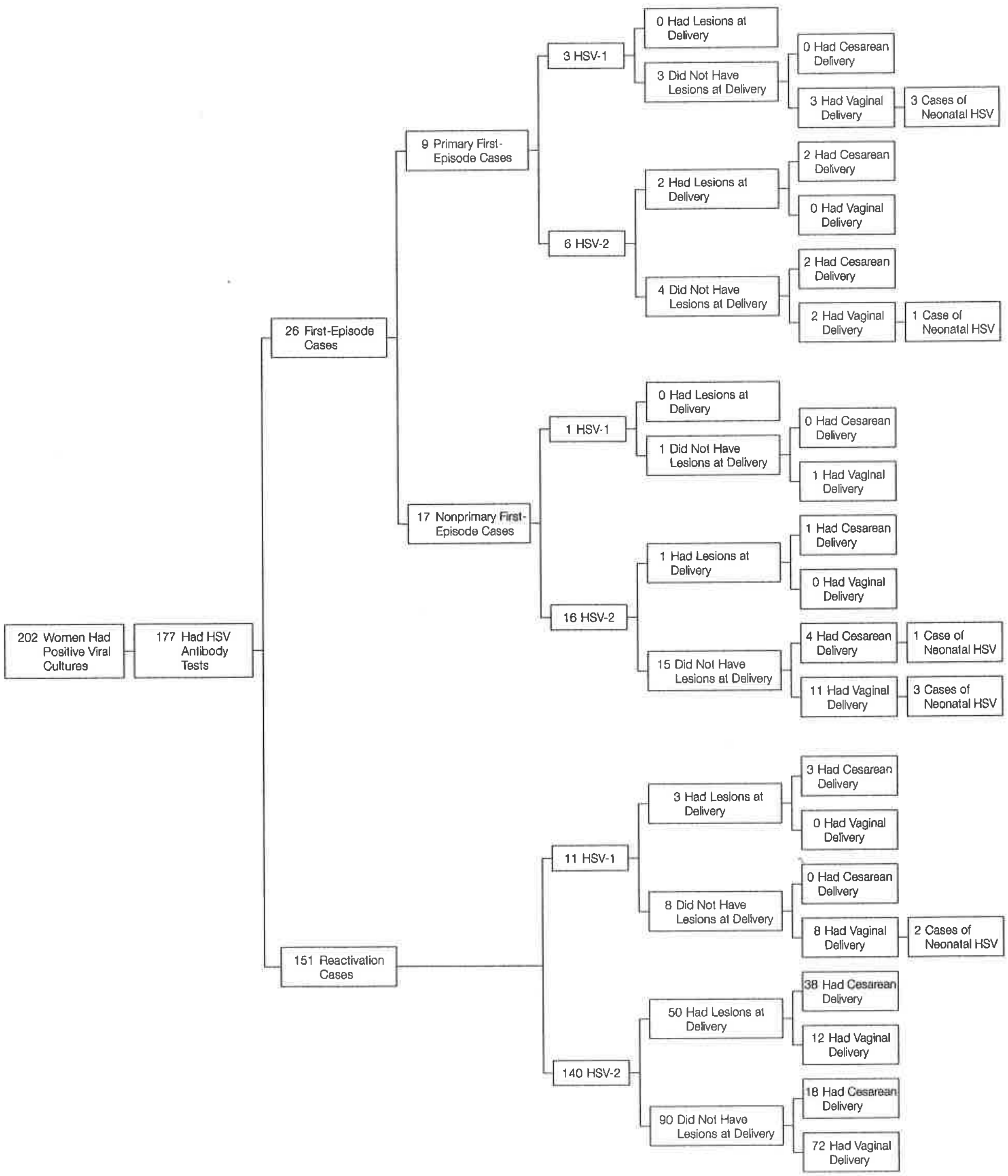
During the study period there were 58362 live births in the study hospitals; 18 cases of neonatal HSV were identified among these live births, for a rate of 1 case of neonatal herpes per 3200 live births. Among the 18 cases, 8 neonates acquired HSV-1 and 10 acquired HSV-2 infection. Of the 10 infants with neonatal HSV-2 infection, 7 were born to mothers with primary or nonprimary first-episode HSV-2 and 3 to mothers with reactivation HSV-2 infection. Of the 8 infants with neonatal HSV-1 infection, 4 were born to mothers with primary HSV-1 and 4 to mothers with reactivation HSV-1.

Herpes simplex virus cultures were obtained within 48 hours of delivery in 40023 (69%) of the 58362 women. Herpes simplex virus was isolated from 202 women (0.5%). Serum samples for HSV antibody status were obtained from 31663, including 177 (88%) of the 202 women with positive cultures (FIGURE). Of these 177 women, 26 had a first-episode genital HSV infection at delivery (3 with primary HSV-1, 6 with primary HSV-2, 1 with nonprimary HSV-1, and 16 with nonprimary HSV-2) and 151 women had reactivation of previously acquired genital HSV (11 with HSV-1, 140 with HSV-2).

Risk Factors for Transmission of Neonatal HSV

Isolation of HSV at delivery from mothers was a major risk factor for neonatal herpes (OR, 346; 95% CI, 125-956; TABLE 1). Neonatal transmission oc-

Figure. Frequency of Neonatal Herpes Simplex Virus (HSV) Among 177 Women Shedding HSV at Delivery



curred in 10 (5%) of the 202 women from whom HSV was isolated. All 3 women shedding HSV-1 due to a primary HSV-1 infection infected their infants, as did 1 (17%) of 6 women with primary HSV-2 infection, 4 (25%) of 16 women with nonprimary first-episode HSV-2 infection, and 2 (18%) of 11 women with reactivation HSV-1 (Figure). None of the 140 women with viral shedding due to reactivation HSV-2 infected their infants.

Table 1. Risk Factors for Development of Neonatal HSV in a Cohort of 40 023 Women With Genital Cultures for HSV Obtained at Delivery

Risk Factors	No./Total (%) of Infants With Neonatal HSV Infection	OR (95% CI)*	P Value†	Adjusted OR (95% CI)‡
Among 40 023 Deliveries With Cultures				
HSV isolated at delivery				
Yes	10/202 (4.95)	346 (125-956)	<.001	. . .
No	6/39 821 (0.02)			
Among 202 Deliveries With HSV Isolated				
Type of delivery				
Cesarean	1/85 (1.2)	0.14 (0.02-1.08)	.047	0.14 (0.02-1.26)
Vaginal	9/117 (7.7)			
Lesions at delivery				
Yes	0/74 (0.0)	0	.01	. . .
No	10/128 (7.8)			
Invasive monitors				
Yes	8/79 (10.1)	6.8 (1.4-32)	.02	3.5 (0.6-19)
No	2/123 (1.6)			
Type isolated				
HSV-1	5/16 (31.3)	16.5 (4.1-65)	<.001	34.8 (3.6-335)
HSV-2	5/186 (2.7)			
First episode				
Yes	8/26 (30.8)	33.1 (6.5-168)	<.001	59.3 (6.7-525)
No	2/151 (1.3)			
HSV isolated from cervix§				
Yes	9/49 (18.4)	32.6 (4.1-260)	<.001	15.4 (1.8-133)
No	1/146 (0.7)			
Premature delivery (<38 wk)				
Yes	6/55 (10.9)	4.4 (1.2-16)	.03	1.7 (0.4-7.6)
No	4/147 (2.7)			
Maternal age, y				
<21	6/56 (10.7)	4.1 (1.1-15)	.03	2.7 (0.6-12)
≥21	4/142 (2.8)			

Abbreviations: CI, confidence interval; HSV, herpes simplex virus; OR, odds ratio.
*ORs and CIs were calculated by logistic regression, except where OR = 0. Where OR = 0, there were no infected infants in 1 of the comparison groups, so logistic regression could not be used.
†P values were calculated from the Fisher exact test.
‡Adjusted ORs were calculated by bivariate logistic regression. The adjusted OR for first episode vs reactivation HSV in the mother is adjusted for viral type isolated. All other adjusted ORs are adjusted for first-episode vs reactivation HSV. Ellipses indicate that adjusted ORs could not be computed.
§HSV isolated from cervix only or cervix and vulva vs HSV isolated from vulva alone.

Table 2. Delivery Route and Acquisition of Neonatal Herpes in Women With Herpes Simplex Virus Isolated From the Genital Tract, Stratified by Presence of Lesions

	Neonatal Infection	No Neonatal Infection	Total
Women with lesions present at delivery			
Cesarean	0	60	60
Vaginal*	0	14	14
Women with subclinical viral shedding			
Cesarean	1	24	25
Vaginal	9	94	103
Overall			
Cesarean	1	84	85
Vaginal	9	108	117
Total	10	192	202

*Lesions noted immediately postpartum or too late for cesarean delivery.

We analyzed the effect of delivery route and presence of genital lesions at delivery on neonatal transmission. Of the 202 women who had HSV isolated from genital secretions, 117 (58%) were delivered vaginally and 85 (42%) underwent cesarean delivery (TABLE 2). Lesions presumed to be caused by genital herpes were the indication for cesarean delivery in 60 (71%) of the cesarean deliveries. Neonatal HSV infection occurred in 1 (1.2%) of 85 cesarean deliveries vs 9 (7.7%) of 117 vaginal deliveries (OR, 0.14; 95% CI, 0.02-1.08; $P=.047$; Table 1). One woman with subclinical nonprimary first-episode HSV-2 infection transmitted HSV-2 to her infant after undergoing a cesarean delivery because of failure to progress 19 hours after rupture of membranes. The protective effect of cesarean delivery appeared to be similar after adjustment for stage of infection (OR, 0.14; 95% CI, 0.02-1.26) or for HSV type (OR, 0.17; 95% CI, 0.02-1.46), although no longer statistically significant.

Genital lesions at delivery were also associated with decreased risk of neonatal herpes among women with HSV isolation. Sixty women had genital lesions and underwent cesarean delivery; an additional 14 women had evidence of genital lesions on retrospective review of the case record. These women were delivered vaginally because their genital lesions were not noted until it was too late to proceed with a cesarean delivery, or immediately following delivery. None of these 74 women infected their infants in comparison with 10 of 128 women who were shedding virus without lesions ($P=.01$; Table 1 and Table 2).

Among 202 women who shed HSV at delivery, 102 had a history of genital herpes and 100 did not. Of the 10 infected infants, 4 were born to mothers with a history and the other 6 to women without a history of genital herpes. Women without a history of genital herpes were more likely to shed HSV subclinically than women with such a

history (87 vs 41; $P<.001$). However, women with a history of genital herpes were more likely to have cesarean deliveries (OR, 5.7; 95% CI, 3.1-11), although this risk was attenuated by adjustment for lesions at delivery (adjusted OR, 2.3; 95% CI, 1.1-4.9).

Among women from whom HSV was isolated, the lack of maternal antibodies to the viral type shed was associated with a marked increase in the risk of transmission to the infant (OR, 33.1; 95% CI, 6.5-168; $P<.001$; Table 1). The increased transmission risk of newly acquired compared with reactivation disease remained statistically significant after adjustment for viral type (OR, 59.3; 95% CI, 6.7-525) and was true both for HSV-2 ($P<.001$) and HSV-1 infection ($P=.08$). In contrast with the protection offered by homologous antibody, heterologous antibody did not protect against HSV transmission (OR, 2.6; 95% CI, 0.5-15) for primary vs nonprimary first-episode infection.

The rate of transmission of HSV from mother to infant was higher when HSV-1 was isolated at delivery (5 [31.3%] of 16) compared with HSV-2 (5 [2.7%] of 186) (OR, 16.5; 95% CI, 4.1-65), and the risk remained significantly elevated after adjustment for newly acquired infection (OR, 34.8; 95% CI, 3.6-335). The risk of transmission was also elevated when HSV was isolated from the cervix vs from the vulva only (OR, 32.6; 95% CI, 4.1-260) and remained statistically significant after adjustment for newly acquired infection. Invasive monitoring, such as fetal scalp electrodes, was noted in 79 (39%) of the 202 women with HSV isolation at delivery and was also a significant risk factor for transmission of HSV (OR, 6.8; 95% CI, 1.4-32). Other risk factors for neonatal HSV were younger maternal age and premature delivery (Table 1), although the adjusted OR for these suggested confounding with newly acquired infection.

Women From Whom HSV Was Not Isolated at Delivery

Cultures were not obtained from 2 of the 18 women who transmitted HSV and negative viral cultures were reported in 6. The viral isolates from the infants and the maternal serum samples were available in all 8 cases. One woman had primary HSV-1, 1 had primary HSV-2, 1 had nonprimary HSV-2, 2 had reactivation HSV-1, and 3 had reactivation HSV-2. We were able to retrieve the specimen obtained for viral isolation at delivery for 2 of the 6 negative cultures. Herpes simplex virus 2 DNA was detected in both.

Table 3. Transmission Rates of Neonatal HSV by Maternal HSV Serologic Status Among Women Who Delivered at the University of Washington and Madigan Army Hospitals

Maternal HSV Serostatus	No./Total (%) of Infants With Neonatal HSV	Rate per 100 000 Live Births (95% Confidence Interval)
HSV seronegative	6/11 115 (0.054)	54 (19.8-118)
HSV-1 seropositive only	6/23 480 (0.026)	26 (9.3-56)
All HSV-2 seropositive	3/13 795 (0.022)	22 (4.4-64)
HSV-2 only	2/5761 (0.035)	35 (4.2-126)
HSV-1 and HSV-2	1/8034 (0.012)	12 (0.3-70)

Abbreviation: HSV, herpes simplex virus.

Risk of Neonatal HSV Infection by Maternal HSV Serologic Status

To evaluate the relationship between maternal HSV serologic status and transmission, we limited our analyses to the 48 390 deliveries at the 2 hospitals where HSV serologic testing was routinely performed. Fifteen of the 18 cases of neonatal herpes were from these hospitals. Among the 31 645 serum samples corresponding to these deliveries, 23% of women were HSV seronegative, 49% had only HSV-1 antibodies, 11% had only HSV-2 antibodies, and 17% had both HSV-1 and HSV-2 antibodies. TABLE 3 shows the estimated rates of neonatal HSV infection computed from these data. The highest (1 in 1900) occurred among women who had no HSV antibodies, whereas the lowest (1 in 8000) was among women who were seropositive for HSV-1 and HSV-2. The small number of observed cases limits the power to detect statistically significant differences among the rates.

COMMENT

Several novel observations about neonatal HSV infection emerged from our analyses. First, while women with all HSV serologic classifications are at risk of transmitting HSV to their infants, the highest risk for transmitting infection to the infant was among HSV seronegative women. This high rate reflects the high efficiency of HSV transmission from seronegative women who acquire primary HSV-1 and HSV-2 and whose infants lack type-specific transplacental antibodies. Second, women with previous HSV-2 infection are at a reduced risk for transmitting HSV-2 to their infants and at essentially no risk of transmitting HSV-1. This reflects the relatively inefficient transmission of HSV-2 in the face of type-specific transplacental antibodies and the seemingly protective effect of genital HSV-2 infection on the acquisition of genital HSV-1 infection. Third, the transmission rate of HSV is highly influenced by management of women in labor, including recognition of lesions, protection offered by cesarean delivery, and maintenance of fetal skin integrity during labor.

Perhaps the most clinically important observation from our study was the finding that cesarean delivery protects against neonatal transmission of HSV. This is the first demonstration of this effect, despite that it has been standard obstetric practice in the United States for 30 years.[5,11,12] Our data, from a cohort study that spans nearly 2 decades of management by various physicians at major service and teaching institutions, provide the first evaluation of this procedure for reducing neonatal HSV. Neonatal herpes occurred less frequently among women with genital lesions than among those experiencing subclinical shedding because women with genital lesions were more

likely to undergo cesarean delivery. Ideally, management practices such as cesarean delivery for genital herpes should be defined by randomized trials. No such trial has been undertaken in the past, and such an attempt is likely to encounter considerable ethical difficulties. Although case series of neonatal HSV show that cesarean delivery is not fully protective,[24,25] our data indicate that it is a rational intervention and should not be abandoned.

Another novel finding was the high efficiency of transmission of HSV-1 from mother to infant, both from primary infection and reactivation of genital HSV-1, among women with genital shedding of HSV. The mechanism of this is unclear but may help explain the increasing frequency of neonatal HSV-1 infection.[26-28]

Development of a strategy to reduce this disease burden seems imperative. While antiviral therapy for neonatal herpes is now available, the morbidity is still high and few inroads in improving time to diagnosis have been made in the last 2 decades.[25,29,30] As such, preventing transmission to the neonate by reducing acquisition of infection in late pregnancy in the mother and altering obstetric management may be the approach most likely to reduce neonatal HSV. Serologic assays that detect antibodies to HSV-1– and HSV-2–specific glycoprotein G1 and G2 are now commercially available[31] and can be used to identify pregnant women who are seronegative for HSV-1, HSV-2, or both and to identify partners who present a potential risk of transmitting infection. These women can be counseled about the importance of avoiding unprotected oral-genital contact or unprotected sex in the last trimester.

Serologic screening for HSV-2 will result in identifying a large number of pregnant women with subclinical HSV-2 infections who are at risk of reactivating HSV-2 at delivery.[17,32-35] Our data indicate that the risk of HSV transmission is low among HSV-2–seropositive women, and routine cesarean delivery is certainly not indicated. Management strategies for HSV-2–seropositive women are complex and need systematic evaluation.[36-38] Potential strategies include suppression of reactivation with antiviral therapy, examination for genital lesions and use of cesarean delivery, and identification of those shedding virus at delivery and intervention in the delivery room, such as cesarean delivery or prophylaxis of the exposed infant with antivirals. Small studies suggest that genital lesions at term may be prevented by long-term daily antiviral therapy in the last month of pregnancy, and such an approach is approved by the American College of Obstetricians and Gynecologists, but some experts still have concerns about the safety of this approach.[11,39,40] The occurrence of false-negative cultures decreases enthusiasm for relying on viral culture alone. DNA amplification techniques offer obvious advantages and have been considered for intrapartum diagnosis of group B streptococcal infections.[41] But the technological issues in conducting and reporting these assays quickly and accurately enough to influence obstetrical management are not trivial. Antiviral, behavioral, and, of course, vaccine approaches to reduce transmission from mother to infant need evaluation in large multi-institutional trials to determine the most effective and economical strategies.

Author Affiliations: Departments of Obstetrics and Gynecology (Dr Brown), Laboratory Medicine (Drs Wald, Morrow, and Corey and Ms Selke), Medicine, Statistics (Dr Zeh), and Epidemiology (Dr Wald), University of Washington, and the Program in Infectious Diseases, Fred Hutchinson Cancer Research Center (Drs Wald and Corey), Seattle.

Author Contributions: *Study concept and design:* Brown, Corey.
Acquisition of data: Brown, Morrow, Selke, Corey.
Analysis and interpretation of data: Brown, Wald, Morrow, Selke, Zeh, Corey.
Drafting of the manuscript: Brown, Wald, Selke, Zeh, Corey.
Critical revision of the manuscript for important intellectual content: Brown, Wald, Morrow, Selke, Zeh, Corey.
Statistical expertise: Zeh.
Obtained funding: Brown, Corey.
Administrative, technical, or material support: Brown, Morrow, Corey.
Study supervision: Brown, Wald, Corey.

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> A chief event of life is the day in which we have encountered a mind that startled us.
> —Ralph Waldo Emerson (1803-1882)

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EXHIBIT F

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Neonatal Herpes Simplex Infection

David W. Kimberlin*

Division of Pediatric Infectious Diseases, The University of Alabama at Birmingham, Birmingham, Alabama 35233

INTRODUCTION

Neonatal infections with herpes simplex virus (HSV) were first reported in the mid-1930s, when Hass described the histopathologic findings of a fatal case (35) and when Batignani reported a newborn with herpes simplex keratitis (14). Over the subsequent decades, the spectrum of disease which HSV can cause in the newborn has been detailed and the efficacy of antiviral therapy in neonatal HSV infections has been established. The earliest antiviral agents with in vitro activity against HSV, including 5-iodo-2′-deoxyuridine and 1-β-D-arabinofuranosylcytosine, proved too toxic in humans to be useful (16, 81). Vidarabine (1-β-D-arabinofuranosyladenine) was the first systemically administered antiviral medication with activity against HSV for which the therapeutic efficacy outweighed its toxicity for the management of life-threatening HSV disease. Licensed for use in the United States in 1977, vidarabine occupies a special place in the historical development of antiviral compounds. Due to toxicity when administered systemically, use of intravenous vidarabine was restricted by the Food and Drug Administration to life-threatening HSV and varicella-zoster virus infections. Multicenter collaborative clinical trials conducted by the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group established its efficacy in the treatment of neonatal HSV infections

* Mailing address: Division of Pediatric Infectious Diseases, The University of Alabama at Birmingham, 1600 Seventh Ave. South, CHB 303, Birmingham, AL 35233. Phone: (205) 996-7802. Fax: (205) 975-9972. E-mail: dkimberlin@peds.uab.edu

(98, 102), HSV encephalitis (101), varicella-zoster virus infections (96), and herpesvirus infections in immunocompromised patients (96, 100). Such collaborative efforts not only established the scientific merit of the compound but also foreshadowed the system by which newer antiviral drugs such as acyclovir and the antiretroviral compounds are evaluated. Additionally, close investigation of vidarabine provided detailed information about herpesvirus infections at a cellular level, illuminating not only the natural history of the diseases but also molecular mechanisms of antiviral action. Intravenous vidarabine has not been available in the United States since 1992, although a topical preparation remains on the market for the treatment of HSV keratitis.

The next development in the management of neonatal HSV disease was a landmark comparison of vidarabine and a lower dose of acyclovir (30 mg/kg/day administered intravenously in three divided doses for 10 days) conducted during the 1980s (92). Most recently, a higher dose of acyclovir (60 mg/kg/day administered intravenously in three divided doses for 21 days) has been evaluated in the management of neonatal HSV disease (47), as discussed in detail below. Each of these trials resulted in a change in the therapeutic standard for the management of neonatal HSV disease, either because of lower toxicity and improved ease of administration (in the case of the lower dose of acyclovir) or because of improved outcome (in the case of the higher dose of acyclovir).

Additional improvements in the outcomes of neonates with HSV disease have been achieved through advances in the diagnostics available to clinicians, the most powerful of which is the application of PCR to patients with neonatal HSV disease (46). This powerful diagnostic tool has enhanced the ability to correctly diagnose neonatal HSV infections and has proven especially beneficial in patients without overt manifestations of HSV disease such as skin vesicles. It also has provided an additional means by which response to therapy can be assessed.

Other areas of promise with respect to prevention of neonatal HSV infections include the development of genetically engineered subunit and live attenuated HSV vaccines for prevention of maternal genital infections and evaluation of antiviral administration to gravid women to reduce the likelihood of clinically apparent genital disease at the time of delivery. Of all the herpesvirus infections, neonatal HSV infection should be the most amenable to prevention and treatment because it is acquired most often at birth rather than early in gestation. This article summarizes the recent developments in neonatal HSV disease management, focusing on enhanced therapeutic interventions, improved diagnostic modalities, and possible means of preventing viral transmission to newborns in the future.

BIOLOGY

Viral Structure

HSV-1 and HSV-2 are two of the eight known viruses which comprise the human herpesvirus family. As with all herpesviruses, they are large, enveloped virions with an icosahedral nucleocapsid consisting of 162 capsomeres, arranged around a linear, double-stranded DNA core. The genome consists of two covalently linked components, designated L (long) and S (short). Each component consists of a unique sequence flanked by inverted repeats. Additionally, the unique L and S components can invert relative to one another, yielding four linear isomers. Each intact HSV virion contains only one of these four isomers, and each of the four is equally virulent (functionally equivalent) in the host cell.

The DNAs of HSV-1 and HSV-2 are largely colinear, and considerable homology exists between the HSV-1 and HSV-2 genomes. These homologous sequences are distributed over the entire genomic map, and most of the polypeptides specified by one viral type are antigenically related to polypeptides of the other viral type. This results in considerable cross-reactivity between the HSV-1 and HSV-2 glycoproteins, although unique antigenic determinants exist for each virus. Viral surface glycoproteins mediate HSV attachment to and penetration into cells and provoke host immune responses. Eleven glycoproteins of HSV have been identified (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM), with a twelfth being predicted (gN). gD is the most potent inducer of neutralizing antibodies and appears related to viral entry into a cell, and gB also is required for infectivity. Antigenic specificity is provided by gG, with the resulting antibody response allowing for the distinction between HSV-1 (gG-1) and HSV-2 (gG-2).

Latency and Neurovirulence

Two biologic properties of HSV which directly influence human disease are latency and neurovirulence. During HSV infection, virions are transported by retrograde flow along axons that connect the point of entry into the body to the nuclei of sensory neurons (82). Viral multiplication occurs in a small number of sensory neurons, and the viral genome then remains in a latent state for the life of the host. With periodic reactivation brought on by events such as physical or emotional stress, fever, UV light, and tissue damage, the virus is transported back down the axon to replicate again at or near the original point of entry into the body. Such reactivation can result in clinically apparent disease (lesions) or clinically inapparent (asymptomatic, or subclinical) infection. The mechanisms by which HSV establishes latency are being intensely investigated but remain incompletely understood.

Neurovirulence refers to the affinity with which HSV is drawn to and propagated in neuronal tissue. This can result in profound disease with severe neurologic sequelae, as is the case with neonatal HSV central nervous system (CNS) disease and with herpes simplex encephalitis in older children and adults. Sites on the HSV genome which mediate this propensity for neurovirulence have been mapped to the thymidine kinase gene as well as the termini of the L component. Of note, the gene identified as $\gamma_1 34.5$ is required for replication in central nervous system tissue and prevents apoptosis of infected neuronal cells. Genetically engineered HSV virions lacking the $\gamma_1 34.5$ gene are currently being investigated as therapies for brain tumors (59, 60).

EPIDEMIOLOGY

Infections in Children and Nonpregnant Adults

Orolabial herpes (HSV-1). Virtually all herpetic orolabial disease is caused by HSV-1. The geographic distribution of

HSV-1 is worldwide, with infection occurring in both developed and underdeveloped countries. Animal vectors for human HSV infections have not been described, and humans remain the sole reservoir for transmission to other humans. Virus is transmitted from infected to susceptible individuals during close personal contact. There is no seasonal variation in the incidence of infection. Because infection is rarely fatal and HSV establishes latency, over one-third of the world's population has recurrent HSV infections and hence, the capability of transmitting HSV during episodes of productive infection. As such, recurrent herpes labialis is the largest reservoir of HSV infections in the community.

Using type-specific serologic assays, the seroprevalence of HSV-1 infections in the United States has been redefined utilizing sera obtained from the randomized National Health and Nutrition Examination Survey (37, 64). By the age of 5 years, over 35% of black children and 18% of white children are infected by HSV-1. Through adolescence, blacks have approximately a twofold higher prevalence of antibodies to HSV-1 than do whites, and females have a slightly higher antibody prevalence than do males. By the age of 40 years, both blacks and whites have a similar prevalence of antibodies, with 70 to 80% being HSV-1 seropositive. A similarly high prevalence of antibodies to HSV-1 exists among persons worldwide, although a high degree of country-to-country variability is seen.

Genital herpes (HSV-2 or HSV-1). Genital herpes infections are caused by HSV-2 or HSV-1. Epidemiologic studies of HSV-2 seroprevalence accurately reflect HSV-2-associated genital disease burden. However, similar studies of HSV-1 seroprevalence are not of equal utility in determining the magnitude of HSV-1-associated genital disease burden, because a majority of adults have acquired HSV-1 orolabial infections by the time they reach adulthood (34), as described above. Seroepidemiologic studies thus cannot distinguish a person with HSV-1 antibody due to prior orolabial infection from someone with prior HSV-1 genital disease.

When a person with no prior HSV-1 or HSV-2 antibody acquires either virus in the genital tract, a first-episode primary infection results. If a person with preexisting HSV-1 antibody acquires HSV-2 genital infection, a first-episode nonprimary infection ensues. Viral reactivation from latency and subsequent antegrade translocation of virus back to the skin and mucosal surfaces produces a recurrent infection.

HSV-2 antibodies do not routinely appear prior to adolescence (37, 56), and antibody prevalence correlates with prior sexual activity. The primary route of acquisition of HSV-2 infections is via genital-genital sexual contact with an infected partner (29, 38, 39, 66). Since the late 1970s, seroprevalence rates for HSV-2 in the United States have increased by 30%, despite concurrent efforts associated with the human immunodeficiency virus epidemic to raise awareness of safer sex practices (32). Currently, one-fifth of U.S. residents aged 12 years or older are infected with HSV-2 and one-quarter of U.S. residents aged 30 years or older are infected with HSV-2. These infection rates and their rise over the past two decades suggest that genital herpes is nearing epidemic proportions. Predictors of positive HSV-2 serologic status include female sex, black race or Mexican-American ethnic background, a greater lifetime number of sexual partners, older age, less formal education, and an income below the poverty line (32, 37, 64). For sexually active Americans with a single lifetime sexual partner, the probability of acquisition of HSV-2 is 10.2%. This figure increases to 20.7, 25.9, 30.9, and 46.1% as the number of lifetime sexual partners increases to 2 to 4, 5 to 9, 10 to 49, and ≥50, respectively (32). Despite these high seroprevalence rates, only 2 to 3% of adults in the United States report ever having had genital herpes, and it is this lack of recognition of one's own infection which contributes to the surreptitious spread of the infection.

Along with the increased incidence of genital HSV-2 infections over the past two decades, there has also been a dramatic rise in the incidence of genital HSV-1 infections. In the early 1980s, approximately 10% of cases of genital herpes in the United States were caused by HSV-1 (17, 26, 51, 64, 73). By the mid-1990s, the percentage of primary cases of genital herpes caused by HSV-1 had doubled to 20% (54). In other parts of the world, HSV-1 accounts for an even larger percentage of genital herpes cases, with rates in excess of 40% reported from Singapore, Sweden, England, Norway, and Japan (13, 25, 57, 65, 75, 87). Genital HSV-1 infections can result from either genital-genital contact or oral-genital contact with an infected person who is actively shedding virus. Given the decreased propensity of HSV-1 to reactivate at the genital site, however, it is oral-genital contact that accounts for most genital HSV-1 infections (54). Whites with genital herpes are more likely than blacks with genital herpes to have infection caused by HSV-1 (54). This may relate to the younger ages at which orolabial HSV-1 infections are acquired among minorities, thereby providing a degree of protection against genital HSV-1 infection in adulthood.

Maternal Genital Infections

Recurrent genital herpes infections are the most common form of genital HSV infections during gestation (94). However, as discussed below, it is the woman with primary genital HSV disease who is at highest risk of transmitting the virus to her baby. About 10% of HSV-2-seronegative pregnant women have an HSV-2-seropositive sexual partner and thus are at risk of contracting a primary HSV-2 infection (53). Among such discordant couples, women who are seronegative for both HSV-1 and HSV-2 have an estimated chance of seroconversion for either virus of 3.7% while women who are already seropositive for HSV-1 have an estimated chance of HSV-2 seroconversion of 1.7% (22). Approximately two-thirds of women who acquire genital herpes during pregnancy have no symptoms to suggest a genital HSV infection (22). This is consistent with the finding that 60 to 80% of women who deliver an HSV-infected infant have no evidence of genital HSV infection at the time of delivery and have neither a past history of genital herpes nor a sexual partner reporting a history of genital herpes (97, 99, 104).

For neonatal transmission to occur in the peripartum period, the gravid woman must be shedding virus, either symptomatically or asymptomatically, at the time of delivery. Studies of nonpregnant HSV-seropositive women have shown that HSV, as detected by PCR, is shed asymptomatically in the genital tract on approximately 1 of every 3 days (90), a remarkable figure that probably has significant implications for both gen-

ital and neonatal spread of HSV infections. Among pregnant women, the incidence of viral excretion proximate to delivery ranges from 0.20 to 0.39% for all pregnant women, irrespective of past history (15, 21, 69). Among pregnant women with a known history of recurrent genital HSV, the incidence of excretion may be as high as 0.77% (89) to 1.4% (4).

Factors influencing neonatal transmission. Factors that influence transmission from mother to neonate include the type of maternal infection (primary versus recurrent) (21, 23, 24, 28, 62), maternal antibody status (24, 70, 104, 105), duration of rupture of membranes (62), integrity of mucocutaneous barriers (e.g., use of fetal scalp electrodes) (24, 41, 67), and mode of delivery (cesarean section versus vaginal) (24).

Infants born to mothers who have a first episode of genital HSV infection near term are at much greater risk of developing neonatal herpes than are those whose mothers have recurrent genital herpes (21, 23, 24, 28, 62). The largest such assessment involved almost 40,000 women without clinical evidence of genital HSV infection and from whom samples were cultured within 48 h of delivery. Of these, 121 women were identified who both were asymptomatically shedding HSV and for whom serum was available for serologic analysis. In this large trial, 57% of babies delivered to women with first-episode primary infection developed neonatal HSV disease compared with 25% of babies delivered to women with first-episode nonprimary infection and 2% of babies delivered to women with recurrent HSV disease (24).

The duration of membrane rupture also appears to affect the risk of acquisition of neonatal infection. A small study published in 1971 demonstrated that cesarean delivery in a woman with active genital lesions can reduce the infant's risk of acquiring HSV if performed within 4 h of membrane rupture (62). Based on this observation, it has been recommended for more than three decades that babies of women with active genital lesions at the time of onset of labor be delivered by cesarean section (3). It was not until 2003, however, that cesarean delivery was definitively proven to be effective in the prevention of HSV transmission to the neonate from a mother actively shedding virus from the genital tract (24). Importantly, neonatal infection has occurred in spite of cesarean delivery performed prior to the rupture of membranes (97).

Incidence of Neonatal Disease

Estimates of the incidence of neonatal herpes have varied from 1 in 3,000 to 1 in 20,000 live births (63). While fluctuations in the incidence of neonatal HSV disease have been observed (21, 63), the current estimated rate of occurrence is approximately 1 in 3,200 deliveries (24). While a progressive increase in the number of cases of neonatal HSV infection has been noted in some areas of the country (85), neonatal HSV infections still occur far less frequently than do genital HSV infections in the adult population of child-bearing age. Overall, the United States, with approximately 4.0 million deliveries per year, has an estimated 1,500 cases of neonatal HSV infection annually.

As discussed above, women who acquire first-episode genital herpes during pregnancy are at far greater risk of transmitting the virus to their newborns than are women with genital reactivation of latent infection. As the baseline prevalence of HSV-2 genital infection increases in the overall population, it will become increasingly likely that a gravid woman may acquire HSV-2 for the first time during her pregnancy through sexual contact with a partner with recurrent or primary genital HSV-2 infection. As such, it is possible that the incidence of neonatal HSV disease may increase in the years to come. Although data on neonatal HSV incidence in very recent years have not been systematically gathered, it is the impression of many experts that the severity of neonatal HSV disease, as manifest by devastating CNS and disseminated infections, has increased over the past 5 years. If confirmed over time, such an observation would probably be related to an increase in the incidence of primary genital infections (32) in pregnant women and its associated increase in the likelihood of transmission to the neonate (24), along with the associated lack of maternal antibodies which can limit the extent of disease in the infected neonate (70).

Times of Transmission to the Neonate and Disease Classifications

HSV disease of the newborn is acquired during one of three distinct time intervals: intrauterine (in utero), peripartum (perinatal), and postpartum (postnatal). The time of transmission for the overwhelming majority (~85%) of infected neonates is in the peripartum period. An additional 10% of infected neonates acquire the virus postnatally, and the final 5% are infected with HSV in utero. HSV infections acquired either peripartum or postpartum can be further classified as (i) disease localized to the skin, eyes, and/or mouth (SEM disease, accounting for ~45% of cases of neonatal HSV); (ii) encephalitis, with or without SEM involvement (CNS disease, accounting for ~30% of cases of neonatal HSV); and (iii) disseminated infection involving multiple organs, including the CNS, lungs, liver, adrenal glands, skin, eyes, and/or mouth (disseminated disease, accounting for ~25% of cases of neonatal HSV). This classification system is predictive of both morbidity and mortality (47, 48, 92, 93, 98). Patients with disseminated or SEM disease generally present to medical attention at 10 to 12 days of life, while patients with CNS disease on average present somewhat later, at 16 to 19 days of life (48).

CLINICAL PRESENTATIONS

Intrauterine Infection

Intrauterine HSV disease occurs in approximately 1 in 300,000 deliveries (10). While rare, in utero disease is unlikely to be missed due to the extent of involvement of affected babies. Infants acquiring HSV in utero typically have a triad of clinical findings consisting of cutaneous manifestations (scarring, active lesions, hypo- and hyperpigmentation, aplasia cutis, and/or an erythematous macular exanthem), ophthalmologic findings (microopthalmia, retinal dysplasia, optic atrophy, and/or chorioretinitis), and neurologic involvement (microcephaly, encephalomalacia, hydranencephaly, and/or intracranial calcification) (33, 36, 40, 61).

TABLE 1. Signs and symptoms prior to study enrollment[a]

Sign or symptom	Incidence in patients with:							
	SEM disease (n = 64)		CNS disease (n = 63)		Disseminated disease (n = 59)		Total (n = 186)	
	No. (%) of patients	Duration (days)[b]	No. (%) of patients	Duration (days)	No. (%) of patients	Duration (days)	No. (%) of patients	Duration (days)
Skin vesicles	53 (83)	3.8 ± 0.5	40 (63)	6.1 ± 1.0	34 (58)	3.7 ± 0.6	127 (68)	4.5 ± 0.4
Lethargy	12 (19)	3.3 ± 0.7	31 (49)	4.6 ± 0.7	28 (47)	3.4 ± 0.7	71 (38)	3.9 ± 0.4
Fever	11 (17)	4.6 ± 1.5	28 (44)	3.1 ± 0.4	33 (56)	4.6 ± 0.6	72 (39)	4.0 ± 0.4
Conjunctivitis	16 (25)	6.5 ± 1.5	10 (16)	4.1 ± 1.3	10 (17)	5.9 ± 1.9	36 (19)	5.7 ± 0.9
Seizure	1 (2)	7.0	36 (57)	2.9 ± 0.5	13 (22)	2.5 ± 0.7	50 (27)	2.9 ± 0.4
DIC[c]	0 (0)		0 (0)		20 (34)	1.5 ± 0.3	20 (11)	1.5 ± 0.3
Pneumonia	0 (0)		2 (3)	9.0 ± 6.0	22 (37)	4.0 ± 0.8	24 (13)	4.5 ± 0.9

[a] Adapted from reference 48.
[b] Duration of symptoms (mean ± SEM).
[c] DIC, disseminated intravascular coagulation.

Disseminated Disease

Historically, disseminated HSV infections have accounted for approximately one-half to two-thirds of all children with neonatal HSV disease. However, this figure has been reduced to about 25% since the development and utilization of antiviral therapy, probably the consequence of recognizing and treating SEM infection before its progression to more severe disease (97). Encephalitis is a common component of this category of infection, occurring in about 60 to 75% of infants with disseminated disease (94). While the presence of a vesicular rash can greatly facilitate the diagnosis of HSV infection, over 20% of neonates with disseminated HSV disease do not develop cutaneous vesicles during the course of their illness (5, 48, 86, 97). Events associated with disseminated neonatal HSV infection which actually result in death relate primarily to the severe coagulopathy, liver dysfunction, and pulmonary involvement of the disease.

CNS Disease

Almost one-third of all neonates with HSV infection are categorized as having CNS disease (with or without SEM involvement) (97). Clinical manifestations of CNS disease include seizures (both focal and generalized), lethargy, irritability, tremors, poor feeding, temperature instability, and bulging fontanelle. Between 60 and 70% of babies classified as having CNS disease have associated skin vesicles at some point in the disease course (48, 86). In infants with CNS disease, mortality is usually caused by devastating brain destruction, with resulting acute neurologic and autonomic dysfunction.

SEM Disease

SEM disease has historically accounted for approximately 18% of all cases of neonatal HSV disease. With the introduction of early antiviral therapy, this frequency has increased to approximately 45% (97). Systematic application of PCR to blood samples from babies with neonatal HSV disease will probably demonstrate that these disease classifications are really more of a spectrum than absolute differences in disease manifestations (11, 30, 50, 58), with SEM disease having more limited viral dissemination but without visceral (liver, lung, etc.) involvement as detected biochemically (e.g., elevated transaminase levels) or clinically (e.g., pneumonitis).

DIAGNOSIS

Overall Evaluation

With ~95% of infected babies acquiring HSV during the peripartum and postpartum periods, therapeutic intervention can potentially occur relatively soon after viral replication begins, prior to widespread viral dissemination and the development of significant and possibly permanent damage to infected tissues and organs (49). For antiviral therapy to be initiated, however, the treating physician must have an index of suspicion which allows for prompt institution of acyclovir therapy. Selected symptoms and signs of neonatal HSV infection at the time of presentation for medical care are presented in Table 1, according to extent of disease. The presence of skin vesicles among patients in any of the disease categories and of seizures in patients with CNS HSV disease appear to be among the findings most suggestive of HSV infection. The absence of fever is common at the time of presentation of neonatal HSV disease. As Table 1 illustrates, however, no single constellation of presenting symptoms and signs identifies all babies with neonatal HSV disease.

A recent comparison between two periods (1981 to 1988 and 1989 to 1997) spanning 16 years suggests that no progress has been made since 1981 in decreasing the time interval between onset of symptoms and initiation of antiviral therapy (48). Given the highly effective antiviral therapies that currently exist for the management of neonatal HSV disease, the most meaningful and immediate manner in which the outcomes of neonatal HSV disease may be rapidly altered is to raise awareness of this infection and hence to decrease the time to diagnostic evaluation for neonatal HSV disease and subsequently to initiation of appropriate antiviral therapy (86). While it is the opinion of the author and of many other experts that acyclovir should not be added routinely to standard antibiotics as management for neonates admitted to rule out sepsis (48), HSV should be considered in the differential diagnosis of acutely ill infants younger than 1 month (48). If the presentation is compatible with neonatal HSV disease, appropriate laboratory specimens should be obtained and acyclovir therapy

should then be initiated. This is especially true if the patient's bacterial cultures are negative at 48 to 72 h and the neonate has not improved clinically. The diagnostic evaluations obtained prior to initiation of acyclovir therapy should include HSV cultures of skin vesicles (if present), oropharynx, conjunctivae, urine, blood, stool or rectum, and cerebrospinal fluid (CSF) (1). Cerebrospinal fluid should also be sent to a reliable laboratory for HSV DNA PCR (46), as discussed below. Liver transaminase levels should also be determined, since their elevation could suggest disseminated HSV infection.

Laboratory Assessment

Serologic testing. Until recently, the commercially available serologic assays were unable to distinguish between HSV-1 and HSV-2 antibodies, severely limiting their utility. In the past few years, type-specific antibody assays have been approved by the Food and Drug Administration and are on the U.S. market. These include tests manufactured by Diagnology (HSV-2) (7, 8, 51) and MRL (now called Focus Technologies; HSV-1 and HSV-2) (55, 68, 103); tests by at least three additional companies are under development. A number of additional tests which claim to distinguish between HSV-1 and HSV-2 antibody are commercially available but have such high cross-reactivity rates that they should be avoided (6). It is important to note that serologic testing identifies only past infection and cannot identify the site of HSV infection: patients with cold sores due to HSV-1 will test HSV-1 seropositive regardless of whether they also have genital HSV-1 infection. With these type-specific assays, however, it is now possible to identify serodiscordant couples in which the woman is HSV-2 seronegative, and the partner is seropositive. Women in such couples are at risk for acquiring primary genital HSV infection during pregnancy and are thus at higher risk of transmitting the virus to their babies during birth. At present the optimal application of these type-specific assays has not been determined. Recent studies documenting the efficacy of condom use (91) and of antiviral suppression of the seropositive partner (L. Corey, S. Tyring, K. Beutner, T. Warren, S. Sacks, R. Patel, A. Wald, G. Mertz, J. Paavonen, and the Valaciclovir Study Group, Program Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother, abstr. LB-3, 2002) in preventing the acquisition of genital herpes suggest that successful interventions based on knowledge of a couple's serologic status can be devised.

In contrast to other congenital and neonatal infections, serologic diagnosis of neonatal HSV infection is not of great clinical value. With the availability of reliable type-specific assays, one barrier to interpreting serologic results in babies with suspected HSV disease has been removed. However, the presence of transplacentally acquired maternal immunoglobulin G still confounds the assessment of the neonatal antibody status during acute infection, especially given the large proportions of the adult American population who are HSV-1 and HSV-2 seropositive. Serial antibody assessment may be useful in the very specific circumstance of a mother who has a primary infection late in gestation and transfers very little or no antibody to the fetus. In general, however, serologic studies play no role in the diagnosis of neonatal HSV disease.

Viral culture. Isolation of HSV by culture remains the definitive diagnostic method of establishing HSV disease. If skin lesions are present, a scraping of the vesicles should be transferred in appropriate viral transport medium on ice to a diagnostic virology laboratory. Such specimens are inoculated into cell culture systems, which are then monitored for cytopathic effects characteristic of HSV replication. Typing of an HSV isolate may then be done by one of several techniques. Other sites from which virus may be isolated include the CSF, urine, blood, stool or rectum, oropharynx, and conjunctivae. Collection of duodenal aspirates for HSV isolation may be indicated in infants with hepatitis, necrotizing enterocolitis, or other gastrointestinal manifestations of disease (95). Specimens for viral culture from multiple body sites (with the exception of CSF) may be combined prior to plating in cell culture in order to decrease costs. The reason is that with the exception of CNS involvement, the important information gathered from such cultures is the presence or absence of replicating virus rather than its precise location.

Of the sites routinely cultured for HSV during a recent study (48), skin cultures and eye or conjunctival cultures consistently provided the greatest yields regardless of disease classification, with ≥90% of cultures being positive. Overall, 58 (94%) of 62 patients had a positive skin or eye culture, 33 (48%) of 69 patients had a positive mouth/oropharyngeal culture, and 17 (40%) of 42 patients with CNS involvement (CNS disease or disseminated disease with CNS involvement) had a positive CSF or brain biopsy culture (48).

PCR amplification. The diagnosis of neonatal HSV infections has been revolutionized by the application of PCR technology to clinical specimens including CSF (2, 46, 50, 58, 76, 77, 88) and blood (11, 30, 50, 58). Direct comparisons of the results of these studies are complicated by differences in the methods used in different laboratories. In the largest series, CSF specimens from 77 neonates in the United States with culture-proven HSV disease were evaluated retrospectively by PCR (46). The results of this analysis both enhanced the understanding of the spectrum of the natural history of neonatal HSV disease and validated the utilization of PCR in the management of such infants. These 77 infants had been previously enrolled during the 1980s in a comparative study of vidarabine and acyclovir for the treatment of neonatal HSV disease. As such, categorization of infants by extent of disease (e.g., SEM disease, CNS disease, and disseminated disease) reflected the laboratory technologies available at the time.

TABLE 2. PCR results from CSF of infected neonates[a]

PCR result	No. (%) of patients with:		
	SEM disease (n = 29)	CNS disease (n = 34)	Disseminated disease (n = 14)
Positive	7 (24)	26 (76)	13 (93)
Negative	22 (76)	8 (24)	1 (7)

[a] Data from reference 46.

As shown in Table 2, HSV DNA was detected by PCR in the CSF of almost one-quarter of infants who had previously been categorized as having SEM disease (46). These results suggest that the spectrum of neonatal HSV disease may reflect more of a continuum than rigid placement in one of three categories, as has been suggested as well by others (58). A complete understanding of the significance of a positive CSF PCR result in an

infant with no other laboratory, radiographic, or clinical evidence of CNS involvement requires additional prospective evaluation.

In the same investigation, HSV DNA was detected in the CSF of 13 (93%) of the 14 infants classified as having disseminated disease (Table 2) (46). Of the 34 infants categorized as having CNS disease, 26 (76%) were PCR positive in their CSF (Table 2). This is remarkably similar to the Swedish experience of applying PCR to stored specimens from patients with neonatal HSV diagnosed between 1973 and 1996, where 78% of neonates with CNS HSV disease were found to be PCR positive from CSF (58). Of the eight neonates with CNS disease and negative CSF PCR results in the U.S. study, seven had a single CSF specimen available for retrospective PCR analysis (46). Furthermore, the specimens for six of the eight infants were obtained 5 days or more after initiation of antiviral therapy, and one could speculate that this time interval could explain why the samples were PCR negative. Thus, the PCR assay in the U.S. investigation had an overall sensitivity of 80% (due to their failure to detect HSV DNA from CSF specimens of eight infants with CNS disease) and an overall specificity of 71% (due to the finding of HSV DNA in the CSF of seven infants with presumed SEM disease) (46). In comparison, the sensitivities of PCR assays used in two other investigations of neonatal HSV disease were 100% (50) and 75% (88) and the specificities were 100% in both studies (50, 88).

While the broad ranges of sensitivity and specificity cited above can be explained at least in part by differences in the methods used in the individual studies, many of which involved retrospective PCR analysis of stored biological specimens, the variability in performance of PCR between laboratories warrants consideration. Interlaboratory standards which ensure that identical specimens processed in two different laboratories will yield identical results are largely nonexistent. Furthermore, the performance of PCR is highly dependent on the manner in which the specimen was collected and maintained prior to reaching the laboratory for PCR analysis (9). Given these caveats, interpretation of PCR results, either positive or negative, must be correlated with the patient's clinical presentation and disease course in determining the ultimate clinical or diagnostic significance of the results. A negative CSF PCR result does not in and of itself rule out neonatal HSV CNS disease.

Given the lack of systematic and large-scale prospective investigation of PCR amplification of CSF specimens in the diagnosis and management of neonates with HSV disease, the clinical significance of positive and of negative CSF PCR results at the end of intravenous therapy has yet to be fully delineated. In the U.S. trial cited above, infants who had HSV DNA detected in the CSF by PCR following completion of intravenous antiviral therapy were more likely to either die or suffer moderate to severe neurologic impairment than were those infants whose post-therapy CSF specimens were PCR negative (Table 3) (46). Differences in disease classifications between the PCR-positive and PCR-negative groups, as well as possible sampling bias (only patients with a clinical indication for repeat lumbar puncture such as persistent seizures, fever, or neurologic deterioration were evaluated) of this retrospective analysis, complicate one's ability to draw definitive conclusions from these findings and further emphasize the need

TABLE 3. PCR results following completion of antiviral therapy[a]

Infant characteristic	No. (%) with PCR result		
	Negative[b]	Positive[c]	P
Disease classification			
CNS disease	4 (36.4)	14 (73.7)	<0.001
Disseminated disease	0 (0.0)	5 (26.3)	
SEM disease	7 (63.6)	0 (0.0)	
CSF indices			
Normal	6 (54.5)	1 (5.3)	
Abnormal	3 (27.3)	17 (89.4)	
Morbidity and mortality after 12 mo			
Normal	6 (54.5)	1 (5.3)	<0.001
Mild	0 (0.0)	0 (0.0)	
Moderate	1 (9.1)	3 (15.8)	
Severe	2 (18.2)	10 (52.6)	
Dead	0 (0.0)	5 (26.3)	
Unknown	2 (18.2)	0 (0.0)	

[a] Adapted from reference 46 with permission of the publisher.
[b] All samples negative after treatment.
[c] At least one positive result.

for prospective data on which informed clinical decisions can be based. Nevertheless, the available data suggest that having HSV DNA detected in CSF at or after the completion of intravenous therapy is associated with poor outcomes (46, 58). All patients with CNS HSV involvement should undergo a repeat lumbar puncture at the end of intravenous acyclovir therapy to determine that the specimen is PCR negative in a reliable laboratory and to document the end-of-therapy CSF indices (48). Persons who remain PCR positive should continue to receive intravenous antiviral therapy until PCR negativity is achieved (46, 48).

TREATMENT AND MANAGEMENT

Antiviral Drugs

Mortality. In the preantiviral era, 85% of patients with disseminated neonatal HSV disease died by 1 year of age, as did 50% of patients with CNS neonatal HSV disease (98) (Table 4). Evaluations of two different doses of vidarabine and of a lower dose of acyclovir (30 mg/kg/day for 10 days) documented that both of these antiviral drugs reduce mortality to comparable degrees (92, 98, 102), with mortality rates from disseminated disease and from CNS disease at 1 year decreasing to 54 and 14%, respectively (92) (Table 4). Despite its lack of therapeutic superiority, the lower dose of acyclovir quickly supplanted vidarabine as the treatment of choice for neonatal HSV disease due to its favorable safety profile and its ease of administration. Unlike acyclovir, vidarabine had to be administered over prolonged infusion times and in large volumes of fluid.

With utilization of a higher dose of acyclovir (60 mg/kg/day for 21 days), 12-month mortality was further reduced to 29% for disseminated neonatal HSV disease and to 4% for CNS HSV disease (47) (Fig. 1 and 2, respectively). Differences in mortality at 24 months among patients treated with the higher and lower doses of acyclovir are statistically significant after

TABLE 4. Mortality and morbidity outcomes among 295 infants with neonatal HSV infection, Evaluated by the NIAID Collaborative Antiviral Study Group between 1974 and 1997[a]

Extent of disease	No. (%) of patients treated with:			
	Placebo (98)	Vidarabine (92)	Acyclovir (92), 30 mg/kg/day	Acyclovir (47), 60 mg/kg/day
Disseminated disease				
Total	13	28	18	34
Dead	11 (85)	14 (50)	11 (61)	10 (29)
Alive	2 (15)	14 (50)	7 (39)	24 (71)
Normal	1 (50)	7 (50)	3 (43)	15 (63)
Abnormal	1 (50)	5 (36)	2 (29)	3 (13)
Unknown	0 (0)	2 (14)	2 (29)	6 (25)
CNS disease				
Total	6	36	35	23
Dead	3 (50)	5 (14)	5 (14)	1 (4)
Alive	3 (50)	31 (86)	30 (86)	22 (96)
Normal	1 (33)	13 (42)	8 (27)	4 (18)
Abnormal	2 (67)	17 (55)	20 (67)	9 (41)
Unknown	0 (0)	1 (3)	2 (7)	9 (41)
SEM disease				
Total	8	31	54	9
Dead	0 (0)	0 (0)	0 (0)	0 (0)
Alive	8 (100)	31 (100)	54 (100)	9 (100)
Normal	5 (62)	22 (71)	45 (83)	2 (22)
Abnormal	3 (38)	3 (10)	1 (2)	0 (0)
Unknown	0 (0)	6 (19)	8 (15)	7 (78)

[a] Adapted from reference 44.

stratification for disease category (CNS versus disseminated) [$P = 0.0035$; odds ratio = 3.3 with 95% confidence interval CI of (1.5, 7.3)] (47). Lethargy and severe hepatitis are associated with mortality among patients with disseminated disease, as are prematurity and seizures in patients with CNS disease (48).

Morbidity. (i) Disseminated and CNS disease. Improvements in morbidity rates with antiviral therapies have not been as dramatic as have improvements in mortality rates. In the preantiviral era, 50% of survivors of disseminated neonatal HSV infections were developing normally at 12 months of age (98) (Table 4). With utilization of the higher dose of acyclovir for 21 days, this percentage has increased to 83% (47) (Fig. 3). In the case of CNS neonatal HSV disease, 33% of patients in the preantiviral era were developing normally at 12 months of age (Table 4) while 31% of higher-dose acyclovir recipients develop normally at 12 months today (47, 98) (Fig. 3). While these differences are not dramatic, it is important to note that as more neonates survive neonatal HSV disease based on the mortality data presented above, the total numbers of patients who subsequently develop normally is higher today even while the percentages of survivors with normal development are not dramatically different. Seizures at or before the time of initiation of antiviral therapy are associated with increased risk of morbidity both in patients with CNS disease and in patients with disseminated infection (48).

(ii) SEM disease. Unlike disseminated or CNS neonatal HSV disease, morbidity following SEM disease has dramatically improved during the antiviral era. Prior to utilization of antiviral therapies, 38% of SEM patients experienced developmental difficulties at 12 months of age (98) (Table 4). With vidarabine and lower-dose acyclovir, these percentages were reduced to 12 and 2%, respectively (92). In the high-dose acyclovir study, no SEM patients developed neurologic sequelae at 12 months of life (47) (Fig. 3).

In the preantiviral era, 70% of neonates with disease initially limited to skin vesicles experienced progression of disease to involvement of the CNS or visceral organs (99). It is likely that the initial reduction in morbidity among patients with SEM disease from 38% (98) to 2–12% (92) resulted from antiviral therapy impeding this progression to CNS or disseminated disease, each of which carries a higher risk of neurologic sequelae (97). The continued reduction in morbidity among patients with SEM disease seen in the recently completed high-dose acyclovir study might relate to a redefinition of what constitutes SEM versus CNS involvement. Prior to the application of PCR technology to neonatal HSV disease, patients were classified as having SEM disease if they had no overt laboratory or clinical evidence of viral dissemination to the viscera and/or CNS. The lack of CNS involvement was manifest by no CNS symptoms (seizures, abnormal neuroimaging studies, abnormal electroencephalograms, etc.) and normal

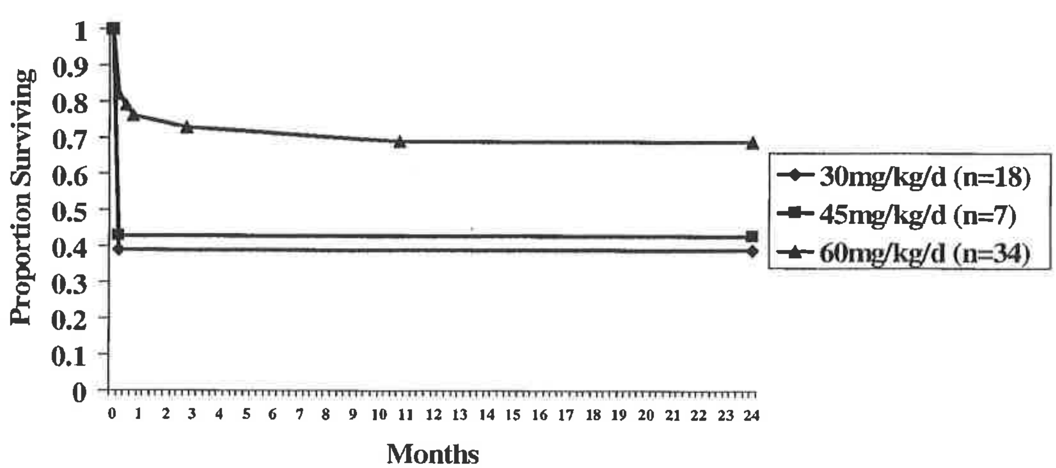

FIG. 1. Mortality in patients with disseminated neonatal HSV disease. Reprinted from reference 47 with permission of the publisher.

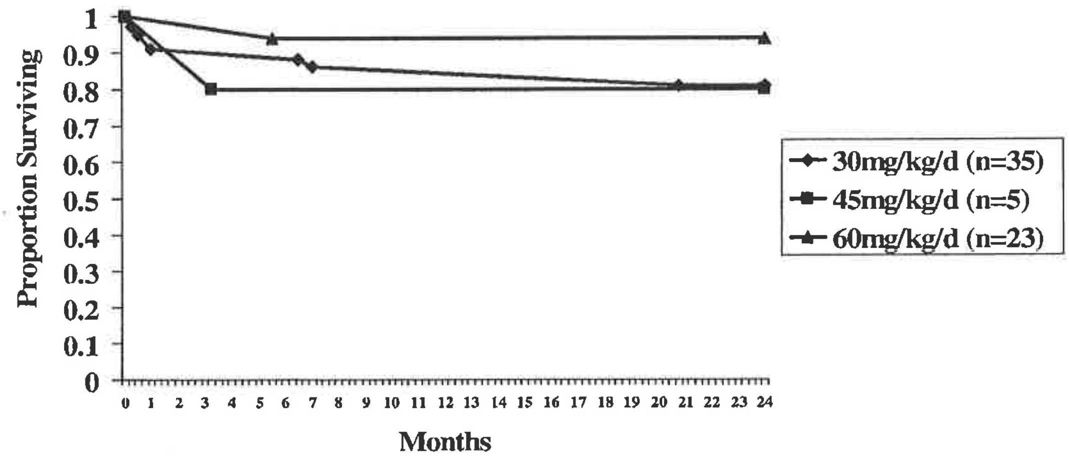

FIG. 2. Mortality in patients with CNS neonatal HSV disease. Reprinted from reference 47 with permission of the publisher.

CSF indices. As discussed above, however, PCR analysis of CSF specimens from neonates classified by these criteria as having SEM disease revealed that approximately one-quarter (7 [24%] of 29) of these infants actually had HSV DNA present in their CSF during the acute disease course (46). One of these seven patients subsequently developed significant neurologic impairment by the age of 12 months. Therefore, it is possible that at least some of the SEM patients in the earlier studies who subsequently developed neurologic impairment actually had subclinical CNS disease, which could be detected only by means of the powerful investigative tool provided in the 1990s by the development of PCR. These data have resulted in a revised classification of CNS disease, such that a positive CSF PCR result is now sufficient to classify a patient as having CNS HSV infection.

Another possible explanation for the neurologic impairment previously experienced by some infants with SEM disease could be that while low-level viremia from the cutaneous lesions results in seeding of the CNS, initial damage to brain tissue during the acute illness does not occur, either due to a host response to infection or due to antiviral therapy. Subclinical reactivation of virus within the CNS, with or without a clinical cutaneous recurrence, might then cause neurologic impairment, as suggested previously (44, 93). Supporting this hypothesis, HSV DNA has been detected in the CSF of an infant with SEM disease at the time of a cutaneous recurrence (42). Randomized, controlled studies of long-term suppressive oral acyclovir therapy following the acute neonatal disease are currently being conducted by the NIAID Collaborative Antiviral Study Group to evaluate this hypothesis. At present, however, no evidence exists to suggest that suppressive oral acyclovir therapy is beneficial in preventing neurological complications. Furthermore, almost half of the infants receiving oral acyclovir in an open-label phase I/II investigation developed neutropenia during therapy (42), raising substantial questions about the safety of such a therapeutic approach outside of the strictly monitored confines of a clinical investigation.

Summary of current treatment. The improvements in mortality and morbidity achieved with the use of higher-dose acyclovir support the use of acyclovir at 60 mg/kg/day delivered intravenously in three divided daily doses, as currently recommended (1, 47). The dosing interval of intravenous acyclovir may have to be increased in premature infants, based on their creatinine clearance (31). The duration of therapy is 21 days for patients with disseminated or CNS neonatal HSV disease and 14 days for patients with HSV infection limited to the SEM (1). As noted above, all patients with CNS HSV involvement should have a repeat lumbar puncture at the end of

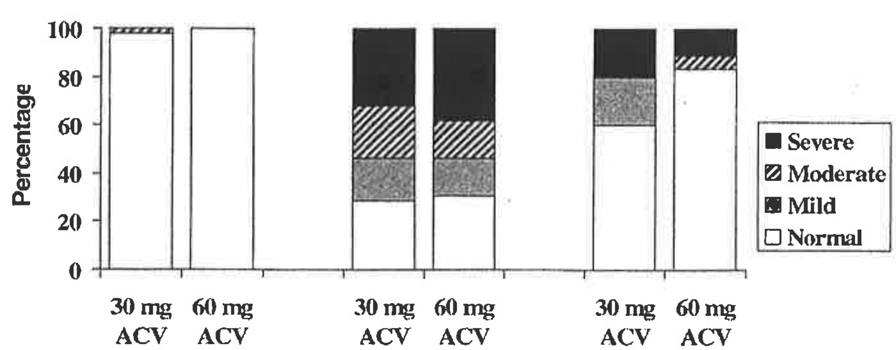

FIG. 3. Morbidity among patients with known outcomes after 12 months of life. ACV, acyclovir. Reprinted from reference 47 with permission of the publisher.

intravenous acyclovir therapy to determine that the specimen is PCR negative as ascertained in a reliable laboratory and to document the end-of-therapy CSF indices (48). Persons who remain PCR positive should continue to receive intravenous antiviral therapy until PCR negativity is achieved (46, 48).

The primary apparent toxicity associated with the use of intravenous acyclovir administered at 60 mg/kg/day is neutropenia, with approximately one-fifth of patients developing an absolute neutrophil count of $\leq$1,000/μl (47). Although the neutropenia resolves either during continuation of intravenous acyclovir therapy or following its cessation, it is prudent to monitor the neutrophil counts at least twice weekly throughout the course of intravenous acyclovir therapy, with consideration being given to decreasing the dose of acyclovir or administering granulocyte colony-stimulating factor if the absolute neutrophil count remains below 500/μl for a prolonged period (47).

Antibody Therapy

Future therapeutic options for further improvement in the management of neonatal HSV disease may reside in the utilization of passive immunotherapy as an adjuvant to active antiviral interventions. Both human and humanized monoclonal antibodies directed against gB or gD are beneficial in animal models of HSV disease (12, 19). Studies with humans have documented the protective effects of high titers of neutralizing antibodies, with neonates with higher neutralizing antibody titers being less likely to become infected with HSV following perinatal exposure (70) and being more likely to have localized disease (and less likely to have disseminated disease) once they are infected (52, 84). While antibody therapy offers promise for improving disease prevention and outcome, studies of humans have yet to be carried out. In addition, an HSV hyperimmune globulin preparation does not exist, and the amount of anti-HSV antibodies present in conventional intravenous gamma globulin preparations is variable. For these reasons, the use of intravenous gamma globulin in the management of neonates with HSV disease cannot be recommended at this time. A monoclonal antibody directed against gD has been produced and may be available for clinical investigation as an adjuvant therapeutic agent by the NIAID Collaborative Antiviral Study Group in future years.

PREVENTION

Cesarean Delivery

As noted above, cesarean delivery in women with active genital lesions can reduce the infant's risk of acquiring HSV (24, 62). In 1999, the American College of Obstetricians and Gynecologists updated its management guidelines for genital herpes in pregnancy (3). To prevent neonatal HSV disease, cesarean section should be performed if genital HSV lesions or prodromal symptoms are present at the time of delivery. As a method to reduce the incidence of neonatal HSV disease, however, cesarean delivery has a number of drawbacks, including the fact that 60 to 80% of babies who develop neonatal HSV disease are born to women without a history of genital herpes (97, 99, 104), and thus infection in these babies may not be prevented by this approach. Furthermore, women with recurrent infections who are shedding virus at the time of delivery are at low risk of their babies developing neonatal HSV disease (21, 23, 24, 28, 62), as discussed above. Decision analyses estimate that 1,580 excess cesarean section deliveries are performed for every poor neonatal outcome prevented, 0.57 maternal death occurs for every neonatal death prevented, and an estimated $2.5 million is spent for every neonatal case averted by this approach (71, 72). These figures contrast with ones regarding cesarean deliveries for women with no history of genital herpes, which result in only nine excess cesarean deliveries per poor neonatal outcome prevented and 0.004 maternal death for every neonatal death prevented. The issue of cesarean delivery is complicated even further by the fact that neonatal infection has occurred in spite of cesarean delivery performed prior to the rupture of membranes (97).

Antiviral Prophylaxis during Pregnancy

Because of the safety record of acyclovir in pregnancy, along with a desire to decrease neonatal HSV disease and reduce the number of cesarean deliveries performed for the indication of herpes, utilization of oral acyclovir near the end of pregnancy to suppress genital HSV recurrences has become increasingly common in clinical practice. Over a 14-year period from 1984 to 1998, the Acyclovir in Pregnancy Registry recorded outcomes of pregnancies in which in utero exposure to acyclovir or valacyclovir occurred (74). No differences were seen with respect to fetal outcomes or birth defects, although the numbers of subjects in the registry were too small to draw definitive conclusions. During the course of this registry, deliberate utilization of acyclovir near the end of pregnancy to suppress genital HSV recurrences became increasingly common in clinical practice, and several small studies investigated the use of acyclovir suppressive therapy during the last weeks of pregnancy (18, 20, 78, 79, 83). These trials suggest that suppressive treatment decreases the occurrence of clinically apparent genital HSV disease at the time of delivery (18, 78, 79), with an associated decrease in cesarean section rates for the indication of genital HSV among women receiving active drug (18, 79, 83). However, they are too small for us to draw definitive conclusions regarding safety and efficacy in treating a disease such as genital herpes which affects one-quarter of the U.S. population. Furthermore, subclinical shedding is not fully suppressed in patients studied to date (20), suggesting that neonatal transmission is likely to still be possible despite antiviral suppression in the mother. Acyclovir concentrations in cord blood of babies whose mothers have received valacyclovir approach levels that have appeared to cause significant neutropenia in infants receiving long-term oral acyclovir suppressive therapy following neonatal HSV disease (42, 43). While neutropenia has yet to be observed among infants born to the small number of gravid women in trials of acyclovir suppressive therapy, ongoing studies continue to investigate this possibility. At present, the safety to the fetus of antiviral suppression in the gravid woman is unproven, and additional studies are needed to more definitively establish the effectiveness and safety of late-pregnancy maternal HSV suppression, including the potential for neutropenia in neonates born to women re-

ceiving antiviral suppressive therapy (D. W. Kimberlin, Abstract SAT 12, Int. J. STD AIDS **13** (Suppl. 1): 60, 2002).

Vaccine Development

A number of efforts have been made to create a vaccine for genital herpes. Until recently, all had been failures. However, a candidate HSV-2 gD subunit vaccine adjuvanted with alum combined with 3-deacylated monophosphoryl lipid A has recently demonstrated promising results. In two large phase III studies, the vaccine has been demonstrated to be safe and, in a subset of volunteers, effective in preventing HSV-1 or HSV-2 genital herpes disease (vaccine efficacy, ~75%) and HSV-2 infection (vaccine efficacy, ~40%) (80). In both studies, efficacy was limited to women who were HSV-1 and HSV-2 seronegative prior to vaccination. There was no evidence of vaccine efficacy in men or in women who were HSV-1 positive but HSV-2 negative prior to vaccination. Because these earlier trials were neither designed nor powered to assess efficacy in women who were HSV-1 and HSV-2 negative, another phase III trial is being undertaken by GlaxoSmithKline and NIAID.

CONCLUSIONS

Tremendous advances in the diagnosis and management of neonatal HSV disease have occurred over the past 30 years. Mortality in patients with disseminated disease has decreased from 85 to 29%, and that in patients with CNS disease has decreased from 50 to 4%. Morbidity has been improved more modestly, with the proportion of patients with disseminated disease who are developing normally at 1 year of age increasing from 50 to 83%. While the proportion of patients with neurologic morbidity following CNS disease has remained essentially unchanged over the past three decades, the total number of patients who are developing normally following HSV CNS disease has increased due to the improved survival. While additional therapeutic advances are possible in the future, more immediate methods for further improvements in outcome for this potentially devastating disease lie in enhancing our awareness of neonatal HSV infection and disease. Educational efforts focusing on these aims and based on an understanding of the biology and natural history of HSV in the gravid woman and the neonate should be systematically undertaken.

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FARLEY

EXHIBIT G

Population-Based Surveillance for Neonatal Herpes in New York City, April 2006–September 2010

Shoshanna Handel, MPH,† Ellen J. Klingler, MPH,† Kate Washburn, MPH,† Susan Blank, MD, MPH,†‡ and Julia A. Schillinger, MD, MSc†‡*

Background: Population-based data for neonatal herpes simplex virus (HSV) infection are needed to describe disease burden and to develop and evaluate prevention strategies.

Methods: From April 2006 to September 2010, routine population-based surveillance was conducted using mandated provider and laboratory reports of neonatal HSV diagnoses and test results for New York City resident infants aged ≤60 days. Case investigations, including provider interviews and review of infant and maternal medical charts and vital records, were performed. Hospital discharge data were analyzed and compared with surveillance data findings.

Results: Between April 2006 and September 2010, New York City neonatal HSV surveillance detected 76 cases, for an average incidence of 13.3/100,000 (1/7519) live births. Median annual incidence of neonatal HSV estimated from administrative data for 1997 to 2008 was 11.8/100,000. Among surveillance cases, 90.8% (69/76) were laboratory confirmed. Among these, 40.6% (28/69) were HSV-1; 39.1% (27/69) were HSV-2; and 20.3% (14/69) were untyped. The overall case-fatality rate was 17.1% (13/76). Five cases were detected among infants aged >42 days. In all, 80% (20/25) of the case-infants delivered by cesarean section were known to have obstetric interventions that could have increased risk of neonatal HSV transmission to the infant before delivery. Over half (68%, or 52/76) of all cases lacked timely or ideal diagnostics or treatment.

Conclusions: Administrative data may be an adequate and relatively inexpensive source for assessing neonatal HSV burden, although they lack the detail and timeliness of surveillance. Prevention strategies should address HSV-1. Incubation periods might be longer than expected for neonatal HSV. Cesarean delivery might not be protective if preceded by invasive procedures. Provider education is needed to raise awareness of neonatal HSV and to assure appropriate testing and treatment.

From the *Public Health Prevention Service, Office of Workforce and Career Development, Centers for Disease Control and Prevention (CDC), Atlanta, GA; †Bureau of STD Control, New York City Department of Health and Mental Hygiene, New York, NY; and ‡Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and Tuberculosis Prevention, CDC, Atlanta, GA

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The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Correspondence: Shoshanna Handel, MPH, NYC Department of Health and Mental Hygiene, BMIRH. E-mail: shandel@health.nyc.gov.

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Infection with herpes simplex virus type-1 (HSV-1) or type-2 (HSV-2) during the neonatal period, or neonatal herpes (neonatal HSV), causes severe morbidity and high mortality rates even when treated.[1,2] The majority of infections (85%) are acquired perinatally, although postnatal (10%) and congenital (5%) infections do occur.[3] There is evidence that an increasing proportion of adult genital HSV infections are attributable to HSV-1[4,5]; however, approaches for preventing neonatal HSV are limited and focused on HSV-2.[1,2,6]

Experts have advocated for making neonatal HSV a nationally notifiable disease; however, neonatal herpes is currently only reportable in a few jurisdictions in the United States (US).[7–10] Estimates of national incidence from other countries range from 1.15/100,000 to 8/100,000 live births.[11–16] Incidence estimates from different parts of the United States are higher, ranging from 8.4/100,000[17] to 69/100,000 live births[9]; this range includes estimates that are not population based, as well as a nationally representative incidence estimate gleaned from a database of pediatric hospital admissions.[18,19,20] Given variability in the prevalence of genital herpes across geographic regions of the United States,[5] variation in incidence of neonatal HSV is expected. Variations are also likely caused by differences in methods used to measure neonatal HSV disease burden. We present findings from a population-based surveillance system for neonatal HSV for the first time in the United States, and compare these findings with analyses of administrative data for the same population.

MATERIALS AND METHODS

In late March 2006, neonatal HSV infection became a reportable disease in New York City (NYC).[21] Clinical laboratories were required to report positive results for HSV on specimens from infants aged ≤60 days who were residents of NYC, and healthcare providers were required to report diagnoses of neonatal HSV infection for the same age group, regardless of whether laboratory results confirmed infection. Certificates of birth, death, and spontaneous termination of pregnancy (fetal death before delivery) were obtained from the NYC Bureau of Vital Statistics for all cases. To identify cases not reported by a provider or laboratory report, a retrospective search of vital records was performed at regular intervals.

The NYC Department of Health and Mental Hygiene investigated reported cases using a standard form. Investigations included confirmation of laboratory testing, telephone interviews with providers involved with each case, review of infant medical records, and maternal labor and delivery records. Interviews with parents were conducted only where

postnatal infection was considered probable. Data collected regarding infant patients included demographics; gestational age; birth weight; circumcision status and date (males only); whether ill at birth; presence and anatomical distribution of lesions; comorbidities; HSV test and its results; acyclovir treatment; cerebrospinal fluid (CSF) and liver function tests and their results; and dates of: first symptom, first seeking medical attention, hospital admission and discharge, specimen collection, diagnosis, and treatment initiation and completion. Data collected regarding infant patients' mothers included demographics, gravidity and parity, history of HSV infection, prenatal HSV serologic testing status, antiviral medication during pregnancy, and presence of genital herpes lesions at delivery. Data collected regarding delivery of infant patients included presentation (vertex or breech), mode of delivery (vaginal or cesarean section), interval between rupture of membranes and delivery, and artificial rupture of membranes or any invasive obstetric procedures.

We defined a confirmed case of neonatal HSV infection as one occurring in an infant aged ≤60 days who tested positive for HSV by culture, direct immunofluorescence assay or other antigen detection test, or polymerase chain reaction. The upper limit for the age range was 60 days to test our hypothesis that some perinatally transmitted cases may not appear until shortly after the neonatal period. We defined a probable case of neonatal HSV as one occurring in an infant aged ≤60 days with no laboratory confirmation of HSV infection, but who had each of the following: (1) a diagnosis of HSV, (2) treatment with acyclovir for ≥7 days, (3) illness clinically compatible with neonatal HSV, and (4) no alternative diagnosis. In NYC, postnatal HSV-1 infections have occurred after ritual Jewish circumcision practices in which the ritual circumciser (mohel) uses his mouth to suck blood away from the incision on the newly circumcised penis.[22] Infection after ritual circumcision was defined as a confirmed case of HSV-1 or untyped HSV, or a probable case, in a male infant who had been circumcised outside of a hospital, with date of illness onset occurring after circumcision; if the date of illness onset was missing, then the date of first specimen collection for HSV testing was used.

Incidence was calculated for infants aged ≤60 days and for infants aged ≤42 days using the number of cases reported during 4.5 years as the numerator. In the denominator, we added three-quarters the number of live births in 2006 plus the number of live births for 2007 to 2009 plus three-quarters the number of live births in 2009 to estimate the number for January to September 2010. Maternal age and race/ethnicity-specific incidence were calculated using maternal age and race-ethnicity data obtained from birth certificates. To obtain a denominator for these incidence calculations, we used a similar method as described earlier and the number of live births by age and race-ethnicity from 2008 to estimate the numbers for 2009 and 2010, since more current data were not available. Case-fatality rates were calculated overall and by viral type.

Pearson chi-square testing was performed by using SAS 9.1 (SAS Institute, Inc., Cary, NC) to identify statistically significant differences in distribution of characteristics among cases with regard to viral type, fatality, infant sex, clinical manifestation, presence of lesions and fever, delivery mode, maternal race, and age at presentation.

We classified cases as follows: skin, eye, or mucous membranes (SEM) infections were those in which herpetic lesions were present or SEM specimens tested positive for HSV with no evidence of central nervous system (CNS), disseminated, or congenital infection. CNS infections were those that were CSF-positive for HSV with no evidence of disseminated or congenital infection. Disseminated infections were those in which there was no evidence of congenital infection, and both aspartate aminotransferase and alanine aminotransferase levels were elevated.[23] Congenital infections were those with signs of HSV-related illness or those from which HSV-positive specimens were collected within 24 hours of birth, or those with stigmata of congenital infection (e.g., microcephaly, microphthalmia, or retinal scarring) noted at birth.

We measured delays in seeking care, diagnosis, and treatment, as well as instances of inappropriate medical treatment. We defined a delay in seeking medical care as >1 day between date of first symptom and date medical care was first sought, a delay in diagnosis as >1 day between date medical care was first sought and date of diagnosis or first specimen collection for HSV testing, and a delay in treatment as >1 day between herpes diagnosis or first specimen collection and beginning treatment with acyclovir. Cases were classified as adequately evaluated if lumbar puncture and liver-function testing were recorded as performed. Inappropriate treatment was defined as administration of less than the recommended course of acyclovir (60 mg/kg/d of intravenous acyclovir for 14 days for SEM cases and 21 days for CNS and disseminated cases); we considered 21 days appropriate therapy for congenital neonatal HSV.[24]

To explain how HSV might have been transmitted despite the protective effect of cesarean delivery, we recorded obstetric factors that might have increased risk for disease transmission before the cesarean delivery. An interval of >4 hours between rupture of membranes and delivery was considered to pose a risk for HSV transmission,[25] as were artificial rupture of membranes, vacuum extraction, and use of fetal scalp electrodes, intrauterine pressure catheters, or forceps.

We used hospital discharge data to measure number of cases of neonatal HSV diagnosed among infants with an NYC zip code of residence who had been discharged from a New York State hospital during January 1997 to December 2008 and who were aged ≤60 days at time of admission, and included any hospital discharges listing an International Classification of Diseases (ICD) Version 9 (ICD-9) code for herpes (codes 054.0–054.9) as the principal, primary, or other diagnosis code. A unique identifier was created by concatenating the encrypted date of birth, sex, and the zip code of the patient's residence to identify infants with more than one hospital discharge listing a herpes ICD-9 code, and only the first such admission was counted. Annual incidence was calculated using annual neonatal HSV hospital discharges as the numerator and annual number of live births in NYC as the denominator.

RESULTS

During the first 4.5 years (April 2006–September 2010) of neonatal HSV surveillance in NYC, 75 reported cases met our case definitions. One additional case was identified by death certificate search, providing 76 cases for analysis. Overall incidence of neonatal HSV was 13.3/100,000 live births or 1/7519 live births; among infants aged ≤42 days, incidence was 12.4/100,000 live births or 1/8065 live births. Among 72/76 (94.7%) cases with information regarding maternal age at delivery, median maternal age was 25 years (range, 16–43 years). Age-specific incidence was highest among infants born to women aged <20 years (47.4/100,000 live births or 1/2110) and declined thereafter (Table 1). Infants born to black non-Hispanic mothers were 1.5 times as likely to be infected with HSV as those born to white non-Hispanic or Hispanic mothers. Black non-Hispanic mothers had the youngest median age at

TABLE 1. Distribution of Cases by Maternal Age and Race/Ethnicity

	All Race/ Ethnicities			Black Non-Hispanic		Hispanic		White Non-Hispanic		Asian		Other/ Unknown	
Maternal Age (yr)	n	Incidence	%	n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
All ages ($P < 0.0001$)	76	13.3	100.0	23	18.0	24	13.2	18	10.4	4	4.9	7	259.3
<20	18	47.4	23.7	10	79.8	2	9.3	2	72.7	0	0.0	4	2,191.8
20–24	21	18.3	27.6	6	19.4	7	14.4	5	21.5	1	8.6	2	353.1
25–29	15	10.1	19.7	4	12.1	5	10.0	3	7.8	2	7.6	1	135.6
30–34	10	6.7	13.2	0	0.0	5	13.5	5	8.7	0	0.0	0	0.0
>34	12	10.1	15.8	3	12.8	5	19.9	3	5.8	1	5.8	0	0.0

delivery (20 years, as compared with 27.5 years for white non-Hispanic and 26 years for Hispanic mothers).

Among the 76 cases, 69 (90.8%) were confirmed and 7 (9.2%) were probable; all had laboratory testing performed. Among the 69 confirmed cases, 28 (40.5%) patients were infected with HSV-1; 27 (39.1%) with HSV-2; and 14 (20.3%) had positive laboratory results that were not type specific. No statistically significant differences between HSV-1 and HSV-2 cases were identified with regard to sex, fatality, clinical manifestation, presence of lesions or fever, delivery mode, or maternal race. In all, 43 (56.6%) of the cases were boys. Of the 13 deaths, 8 (61.5%) were among girls; 9 (69.2%) occurred within the first 2 weeks of life (Table 2). Although not statistically significant, the fatality rates differed by HSV type (21.4% among HSV-1 cases and 18.5% among HSV-2 cases). Most of the cases (56.5%) were SEM; 23.2% were disseminated, 17.4% were CNS infections, and 2.9% were congenital infections. Lesions were present among 41 (60.3%) of the 68 cases for which lesion data were available. Fever was present among 19 (31.1%) of the 61 cases for which data were available. Among the 61 cases with known fever and lesion data, 19.7% had neither fever nor lesions (Table 3). In all, 27 (69.2%) SEM cases had lesions noted, compared with 5 (41.7%) CNS cases, 7 (43.8%) disseminated cases, and both (100%) of the congenital cases.

Four (9.3%) of the 43 male patients met the definition for infection after ritual Jewish circumcision. All 4 case patients had lesions on the penis or the scrotum (2 on the penis only, 1 on the scrotum only, and 1 on both the penis and the scrotum); 3 of the 4 case-patients were laboratory-confirmed HSV-1 cases. The interval between circumcision and illness onset ranged 2 to 12 days (median, 3.5 days).One of the case-patients had CNS infection, the remaining 3 had SEM disease.

Of all cases, 56 (73.7%) were diagnosed at age ≤14 days; 12 (15.8%) at age 14 to 30 days; 3 (3.9%) at age 31 to 42 days; and 5 (6.6%) at age 43 to 60 days. Case-patients diagnosed at age ≤14 days had a higher fatality rate than those diagnosed at age ≥15 days (21.4% vs. 5%; $P = 0.094$). Of the 5 cases diagnosed among infants >42 days, 2 were HSV-1 (delivered by cesarean section); 2 were HSV-2 (one vaginally, and the other with unknown mode of delivery); and 1 was a probable case (cesarean section). Among the 57 case mothers for whom we had data, 11 (19.3%) had a known history of HSV, and 5/52 (9.6%) of those for whom data were available

TABLE 2. Characteristics of Fatalities

Sex	HSV Type	Syndrome	Mode of Delivery	Obstetric Risk Factors	Maternal History of HSV	Age at Diagnosis (in Days)	Age at Death (in Days)	HSV Indicated on Death Certificate
Male	1	Disseminated	Cesarean	Yes*†	Unknown	7	12	No
Female	1	SEM	Vaginal	Unknown	Unknown	N/A	0	No
Female	1	Disseminated	Vaginal	Yes†	No	8	5	Yes‡
Female	1	Disseminated	Cesarean	Yes†§	Unknown	8	14	Yes‡
Male	2	Disseminated	Cesarean	Unknown	Unknown	11	11	Yes¶
Male	2	Disseminated	Cesarean	Yes†‖	No	6	12	No
Male	2	Disseminated	Cesarean	Yes†	No	5	8	Unknown
Male	1	SEM	Cesarean	No	No	14	20	Unknown
Female	Unknown	Congenital	Cesarean	Yes§	No	0	3	Unknown
Female	Unknown	Disseminated	Cesarean	No	No	10	23	Yes¶
Female	1	Disseminated	Vaginal	Yes§	No	8	11	Unknown
Female	2	Disseminated	Cesarean	Yes†§	No	12	15	Unknown
Female	2	Disseminated	Vaginal	Yes†§**	No	16	29	Unknown

*Internal monitor.
†Prolonged rupture of membranes.
‡Underlying cause.
§Artificial rupture of membranes.
¶Immediate cause.
‖Intrauterine pressure catheter.
**Vaccuum extraction.

TABLE 3. Characteristics of Case Infants and Their Births, by Viral Type

	All Cases		Confirmed Cases							Probable Cases	
			Untyped		HSV-1		HSV-2		*P* (HSV-1 vs. HSV-2)		
	N	%*	n	%*	n	%*	n	%*		n	%*
Total	76	100%	14		28		27			7	
Deaths (case-fatality rate)	13	17.1	2	14.3	6	21.4	5	18.5	0.787	0	0
Sex (n = 76)											
Male	43	56.6	8	57.1	16	57.1	13	48.1	0.504	6	85.7
Female	33	43.4	6	42.9	12	42.9	14	51.9		1	14.3
Mean/median age at diagnosis, in days (n = 76)	12.5/9.5		7.9/8.0		13.8/9.5		13.6/11.0		0.957	11.9/7.0	
Clinical manifestation (n = 69)			n = 13		n = 27		n = 23			n = 6	
SEM	39	56.5	9	69.2	17	63.0	8	34.8	0.135	5	83.3
CNS	12	17.4	1	7.7	3	11.1	7	30.4		1	16.7
Disseminated	16	23.2	2	15.4	7	25.9	7	30.4		0	0
Congenital	2	2.9	1	7.7	0	0	1	4.4		0	0
Lesions present (a case can have lesions in multiple sites) (n = 68)			n = 13		n = 27		n = 22			n = 6	
Yes—head	20	29.4	2	15.4	8	29.6	7	31.8	0.951	3	50.0
Yes—trunk	13	19.1	3	23.1	4	14.8	4	18.2	0.804	2	33.3
Yes—genitals/buttocks	13	19.1	5	38.5	4	14.8	1	4.5	0.219	3	50.0
Yes—extremities	12	17.6	4	30.8	2	7.4	6	27.3	0.073	0	0
None	27	39.7	3	23.1	15	55.6	9	40.9	0.308	0	0
Fever present (n = 61)			n = 13		n = 25		n = 18			n = 5	
Yes	19	31.1	2	15.4	8	32.0	9	50.0	0.234	0	0
No	42	68.9	11	84.6	17	68.0	9	50.0		5	100.0
Delivery mode (n = 72)			n = 13		n = 28		n = 24			n = 7	
Vaginal	45	62.5	10	76.9	17	60.7	14	58.3	0.862	4	57.1
Cesarean	27	37.5	3	23.1	11	39.3	10	41.7		3	42.9
Obstetric risk factor† (n = 63)			n = 13		n = 25		n = 19			n = 6	
Yes	52	82.5	11	84.6	22	88.0	13	68.4	0.111	6	100.0
No	11	17.5	2	15.4	3	12.0	6	31.6		0	0
Maternal genital lesions at delivery (n = 52)			n = 12		n = 19		n = 16			n = 5	
Yes	5	9.6	1	8.3	2	10.5	1	6.3	0.653	1	20.0
No	47	90.4	11	91.7	17	89.5	15	93.7		4	80.0

*Column percentages.
†Obstetric risk factors include the following: rupture of membrane >4 h preceding delivery, artificial rupture of membrane, and invasive monitoring or procedures.
HSV indicates herpes simplex virus; SEM, skin, eye, and mucous membrane infection; CNS, central nervous system infection.

had lesions at delivery. None of the 8 cases diagnosed after 30 days of age were born to a mother with a known history of HSV or acyclovir use during pregnancy.

We found a delay in seeking care for 12/59 (20.3%) cases (median: 2 days; range: 2–10 days), a delay in diagnosis for 26/66 (39.4%) cases (median: 4.5 days; range: 2–21 days), and a delay in initiating acyclovir treatment for 18/61 (29.5%) cases (median: 3 days; range: 2–18 days). Overall, 38/54 (70.4%) cases with complete information with which to judge delays had one or more delays. Of the 38 cases where there were delays, 12 (31.6%) had fever, 27 (71.1%) had lesions, and 4 (10.5%) had neither fever nor lesions. Of 66 liveborn infants with complete information regarding lumbar puncture, 57 (86.4%) received lumbar puncture with HSV testing. Of 63 infants, 50 (79.4%) with available information had liver-function tests performed. Only 19 (51.4%) of the 37 patients for whom we had data related to treatment had received an appropriate acyclovir regimen; all of these had received an adequate evaluation. Over half (68%, or 52/76) of all cases lacked timely or ideal diagnostics or treatment.

Length of hospitalization was calculated for 61/76 (80.3%) cases; median was 15 days and varied with clinical manifestation—disseminated cases, median was 11 days (range, 2–39); SEM cases, median was 15 days (range, 0–86 days); and CNS cases, median was 22 days (range, 10–46). The 2 congenital cases were hospitalized for a median of 40.5 days (range, 3–78).

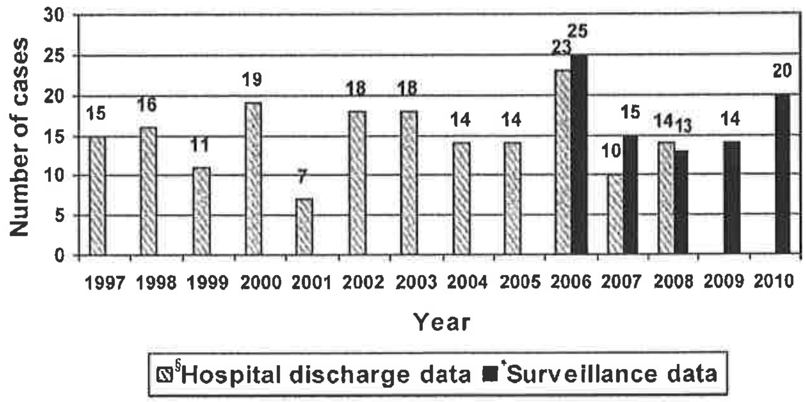

Figure 1. NYC resident neonatal herpes cases identified using an administrative data set of discharges from New York State (including New York City) hospitals during 1997–2008, compared to those reported to New York City through routine public health surveillance during 2006–2010. §Hospital discharge data for 2009 and 2010 are not yet available. *For 2006, and for 2010, the total number of cases was estimated by annualizing 9 months of reported cases.

Where mode of delivery was known, 37.5% (27/72) of the infants were delivered by cesarean-section. Among the 25 cases delivered by cesarean for whom we had data related to obstetric risks for HSV transmission, 20 (80.0%) had at least one such risk. (17 had >4 hours between rupture of membranes and delivery, 10 had artificial rupture of membranes, 5 had invasive instrumentation including vacuum extraction, fetal scalp electrodes, intrauterine pressure catheters, or forceps.) Only 2 of the cesarean deliveries were performed because of a perceived risk of HSV transmission. In both cases, the mother had a known history of genital HSV, and active genital lesions were noted at delivery. Among 45 cases delivered vaginally, 31 (68.9%) had at least one known obstetric risk for neonatal HSV transmission. (20 had >4 hours between rupture of membranes and delivery; 16 had artificial rupture of membranes; 12 had invasive instrumentation including vacuum extraction, fetal scalp electrodes, intrauterine pressure catheters, or forceps.)

Administrative Data Findings

During the 12-year interval from 1997 through 2008, a total of 179 infants were discharged with an ICD-9 code for herpes after an admission at age ≤60 days; 84/179 (46.9%) were male. Only 20/179 (11.2%) infants had been admitted at age >42 days. Median duration of admission was 14 days. During 1997 to 2008, annual incidence of neonatal HSV ranged from 5.6/100,000 live births (in 2001) to 18.3/100,000 live births (in 2006); median annual incidence was 11.8/100,000 live births. For infants aged ≤42 days, incidence ranged from 4.8/100,000 live births (in 2001) to 15.1/100,000 live births (in 2006); median incidence was 11.0/100,000 live births (Fig. 1).

DISCUSSION

We present the first population-based surveillance findings for neonatal HSV in the United States, as well as a comparison with findings from an administrative data set for the same population. Both methods yielded similar incidence rates, and were within the range of previously reported estimates. Our findings provide insight into neonatal HSV epidemiology. Laboratory-confirmed cases were diagnosed well after the first 30 days of life, and these included HSV-2 infections, suggesting a longer-than-expected incubation period. Our findings also reveal a substantial proportion of cases attributable to HSV-1.

The similarity in incidence estimates gleaned from NYC surveillance, and administrative data indicate that the latter may provide a reasonable means of measuring HSV disease burden in jurisdictions without resources to implement neonatal HSV surveillance. However, administrative data are often untimely and therefore do not allow for a public health response to epidemiologic findings. Additionally, administrative data can be difficult to deduplicate, rely on ICD-9 codes that are not specific to neonatal HSV, and often lack detailed clinical and laboratory information, thereby limiting accuracy and utility.

Disparities in risk for neonatal HSV by maternal age and race/ethnicity were apparent in our findings. Younger mothers might be less likely to be infected with HSV at the start of a pregnancy and at increased risk for acquiring HSV during pregnancy. Moreover, because genital HSV-2 infections are particularly prevalent among black non-Hispanic New York residents,[26] they might be more likely than women of other races/ethnicities to be exposed to HSV.

Our findings differed in several ways from those reported by other North American investigators. We found a lower proportion of CNS cases (17.4%, as compared to 30%) and a higher proportion of SEM cases (56.5%, as compared to 45%) than previously reported.[3] The former was surprising, especially because highly sensitive nucleic-acid amplification tests are increasingly being used to test CSF specimens,[27,28] and the majority of our cases (76.0%) had CSF testing. However, our findings on distribution of cases by clinical manifestation was similar to what was found in Canadian surveillance.[11] Our findings on prevalence of fever (31.1%) was also similar to what has been previously reported.[29] We also found a higher case-fatality rate among disseminated cases (62.5%) than previously reported (29%), but no fatalities among CNS cases, in contrast to previous reports of fatality rates of 4% to 15%[2,29] among CNS cases. These findings may be explained, at least in part, by our use of a definition for disseminated disease which selects for only very severe disease and by the increasing use of highly sensitive tests (polymerase chain reaction) to test CSF, which may classify as CNS disease cases who might have been considered SEM in the past.

Over one-third of the reported case-patients had been delivered by cesarean section, suggesting that the protective effect of cesarean delivery can be undermined when other obstetric risk factors for transmission have already occurred. Because a majority of neonatal HSV cases were among infants born under circumstances that would not prompt provider suspicion of risk for HSV infection, opportunities for intervention are limited. Prenatal screening of pregnant women and their sex partners could enable providers to counsel seronegative women with seropositive partners about abstinence or safer sex during pregnancy,[17] or to recommend acyclovir suppressive treatment during the third trimester to HSV-positive women,[30–32] but

both these strategies are unproven, expensive, and carry risks (of undue strain on the woman's relationship and possible toxicity to the infant,[1] respectively).

Postpartum infections could be reduced by educating parents and caregivers about ways to avoid transmitting infection. Unfortunately, it is difficult to modify the practice of ritual Jewish circumcision with oral suction because of the religious value attached to it by certain sects.[33] A vaccine for HSV would be the best prevention strategy, but the HSV vaccine in Phase III trials has recently proven ineffective.[6] To prevent the majority of neonatal HSV cases, a vaccine would have to be effective against both HSV types and be administered before sexual debut.

Opportunities to intervene in the progression of disease were missed, evidenced by delays in diagnosis for over 1/3 of cases and delays in initiating antiviral treatment in nearly 1/3 of cases. A majority (89.5%) of those cases where delays in care seeking, diagnosis, and/or treatment were present had fever or lesions, which may support the case for increased caregiver and provider education. Nonspecific presentation, like the 19.7% of cases we found with neither fever nor lesions, does make diagnosis of neonatal HSV difficult, so pediatric providers should be encouraged to consider neonatal HSV in the differential diagnosis of ill infants, to perform SEM testing, lumbar puncture, and liver function tests, and to initiate intravenous acyclovir treatment immediately when neonatal HSV is suspected.

Our study has several limitations. It is likely that neonatal HSV cases were underreported and those reported might be biased toward more severe disease. The relatively limited number of cases limits our ability to make definitive statistical comparisons among our cases and to those reported in other case series and makes certain statistical analyses unstable. Due to missing information on some cases, there may be some misclassification of disease syndrome; however, that is most likely to have resulted in an overestimate of SEM cases. We lack data concerning lumbar punctures performed at the end of treatment; therefore, we were unable to assess whether follow-up treatment was performed when needed. Length of hospitalization for neonatal HSV might have been overestimated because it includes hospitalization for non-HSV illness, and might appear misleadingly short for disseminated cases, which are more likely to result in death. The number of congenital cases might have been overestimated because we may have included infants' ill at birth with conditions other than neonatal HSV who were colonized with HSV, which might have cleared without treatment. Finally, some of our findings may not be generalizable outside of NYC. For example, the incidence is affected by the prevalence of genital HSV in the population, which varies. However, some of our findings (e.g., delays in diagnosis, treatment, and seeking care, and case fatality rates) are likely to be generalizable.

CONCLUSION

Administrative data may provide an adequate and inexpensive means to assess local neonatal HSV burden, although such data lack the detail and timeliness of surveillance data. We believe routine surveillance for neonatal herpes is of value; our data provide new insights, give a baseline incidence from which to evaluate the impact of future prevention efforts, and point to the need for parental and provider education regarding neonatal HSV. Challenges remain for reducing incidence of neonatal HSV, as all current prevention strategies are limited.

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